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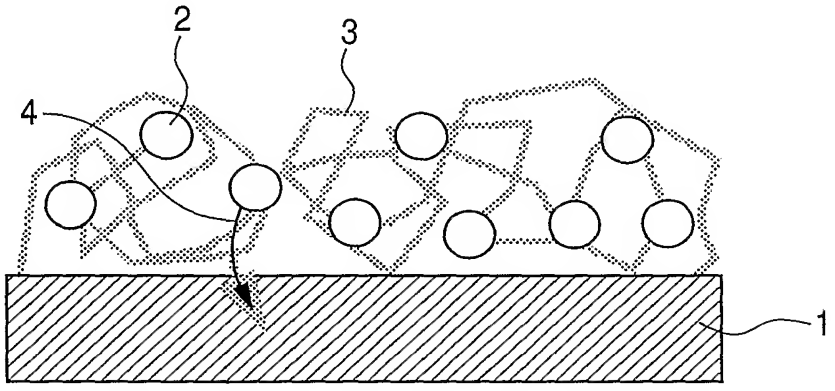
(54) **Title:** ENZYME ELECTRODE, AND DEVICE, SENSOR, FUEL CELL AND ELECTROCHEMICAL REACTOR EMPLOYING THE ENZYME ELECTRODE



(57) **Abstract:** An enzyme electrode has a conductive member and an enzyme, wherein the conductive member has a porous structure, and the enzyme is immobilized through a carrier in pores constituting the porous structure. An enzyme electrode device, comprises the enzyme electrode, and wiring connected to the conductive member of the enzyme electrode.

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DESCRIPTION

ENZYME ELECTRODE, AND DEVICE, SENSOR, FUEL CELL AND ELECTROCHEMICAL REACTOR EMPLOYING THE ENZYME ELECTRODE

TECHNICAL FIELD

The present invention relates to an enzyme electrode. More specifically, the present invention relates to an enzyme electrode having a carrier and an enzyme immobilized on an electroconductive member having voids. The present invention relates further to a process for producing the enzyme electrode, a device employing the enzyme electrode, and uses thereof.

BACKGROUND ART

An enzyme, a proteinaceous biocatalyst formed in a living cell, is highly active under mild conditions in comparison with ordinary catalysts. Further, the enzyme is highly specific to a substrate undergoing an enzymatic reaction, and catalyzes a specific reaction of a specific substrate. Ideally, the enzyme having such properties will enable preparation of a highly selective electrode having a low overvoltage for a redox reaction on the electrode. However, the active centers of most redox enzymes

(oxidoreductases) are usually enclosed in a deep interior of a three-dimensional structure of glycoprotein, so that direct high-speed electron transfer is difficult between the oxidoreductase and the electrode. To cancel the difficulty, a method is disclosed which connects electronically the enzyme with the electrode with interposition of a substance called a mediator. The connection of the oxidoreductase with the electrode through the mediator enables control of an enzymatic reaction by the electrode potential and performance as an energy conversion element. In particular, a device called a biofuel cell has a feature of a biological catalyst, unlike an ordinary fuel cell employing a metallic catalyst like platinum: in principle, any substrate utilized by a living body can be used in the biofuel cell, including sugars, alcohols, amines, and hydrogen on a negative electrode; and oxygen, nitrate ions, and sulfate ions on a positive electrode. In the early stage of the development, the enzyme and the mediator are dissolved in an electrolyte solution for simplicity of the experiment system and for freedom of the transfer thereof. Later, methods of immobilization thereof on the electrode are disclosed for improvement of the efficiency, prevention of leakage into the system, and continuous and long-term use of the electrode. In one method, a carrier is

used for immobilizing an enzyme and a mediator on a conductive member. Generally, chemical or electrostatic immobilization of an enzyme and a mediator on a carrier retains effectively the enzyme and the mediator in comparison with immobilization by physical adsorption, preventing leakage out of the system, and enabling repeated use of the enzyme electrode.

An index of the performance of the enzyme electrode is an electric current density, which is an electric current intensity relative to a projected area of a conductive member. The higher current density enables improvement in detection sensitivity, simplification of a measurement portion, and miniaturization of detector portion when used in a sensor based on current intensity detection; improvement of output when used as an electrode of a fuel cell; and shortening of a reaction time when used as an electrochemical reactor, advantageously. The current density of the enzyme electrode can be increased by increase of a turnover number (a number of substrate molecules converted by an enzyme in a unit time), improvement of electron transfer rate and efficiency between the mediator and the electrode, the enzyme-holding density (the amount of the enzyme per projected area of the conductive member), and so forth.

A typical method for immobilization by use of a carrier is an entrapping immobilization (Fig. 1). In this method, an enzyme is entrapped in a carrier such as a polymer, and the carrier is immobilized on a surface of a conductive member. Fig. 1 is a sectional view showing schematically an entrapping immobilization of an enzyme. In Fig. 1, enzyme 2 is immobilized by entrapping in a layer of carrier 3 on a base plate 1 constituted of a conductive member to cause an electric charge flow as shown for example by the numeral 4. In this entrapping immobilization method, the charge formed by an enzyme/substrate reaction is taken out by the mediator in the carrier, transferred by electron hopping between the mediator molecules to the vicinity to the conductive member, and finally detected by transfer of the electric charge between the mediator and the conductive member. Generally, simple increase of amount of the enzyme on the carrier for increase of the enzyme held by the carrier for the projected area of the conductive member will lower the electron transfer rate between the enzyme/carrier, so that the increase of the current density is limited. In contrast, in the entrapping immobilization employing a mediator, even when the enzyme is immobilized in a density higher than the value of the enzyme occupation area divided by effective surface area of the conductive member,

the electric charge can be transferred between the electrode and the enzyme through the carrier. Therefore, by increasing the amount of the immobilized enzyme and increasing the thickness of the carrier layer, the enzyme immobilization density per projected area of the conductive member (the amount of the immobilized enzyme in the carrier-containing layer) can be increased. Generally, however, since electron diffusion is slow in the carrier-containing layer, the velocity of electron diffusion through the carrier is limited and the electric charge transfer efficiency is lowered at a carrier-immobilized enzyme layer larger than a certain thickness. Therefore the carrier immobilization layer thickness is preferably less than a certain limit, so that the increase of the current density by increase of the immobilized enzyme per projected area of the conductive member is limited. A use of enzyme electrode utilizing the entrapping immobilization for a fuel cell is disclosed in U.S. Patent No. 6,531,239 (Heller et al.) in which the enzyme electrode is prepared by immobilization of an enzyme by a polymer containing an mediator in the molecule.

The enzyme immobilization density can be increased effectively by increasing the effective surface area of the electrode. A typical method

therefore is physical adsorption of an enzyme on a conductive member composed of a carbonaceous material particles and a binder polymer (Fig. 2). In Fig. 2, the enzyme electrode has a layer in which enzyme 2 is immobilized by use of binder polymer 6 on particulate carbon 5, the layer being placed on the surface of base plate 1. In this enzyme electrode, for example, electric charge can flow through particle boundaries 7 of carbon particles 5 as indicated by arrow mark 4. In this enzyme electrode, the resistance at contact point 7 between the carbon particles is high, and the total resistance increases with the thickness of the conductive member to increase the internal resistance of the enzyme electrode to lower the performance of the enzyme electrode. Therefore, the conductive member is preferably used in a thickness smaller than a certain thickness, which limits the increase of the current density by increase of the amount of the immobilized enzyme per projection area of the conductive member (increase by enlargement of the effective surface area of the electrode).

Furthermore, in this enzyme electrode, no carrier is used differently from the entrapping immobilization electrode, resulting in low enzyme-retaining ability and limitation in repeated use of the enzyme electrode. Such an enzyme electrode is disclosed in U.S. Patent No. 4,970,145 (Bennetto et al.) in which

the conductive member is formed by immobilizing the carbon particles and the platinum-type metal particles together by a resin.

DISCLOSURE OF THE INVENTION

In the aforementioned entrapping immobilization, the amount of the immobilized enzyme can be increased by increasing the thickness of the enzyme immobilization layer in which method an enzyme is immobilized in a layer containing a carrier without impairing the electronic connection between the enzyme and the conductive member. Generally, however, since the carrier has a low electron diffusion coefficient, the charge transfer efficiency drops above a certain thickness of the enzyme-immobilization layer. Therefore, the enzyme immobilization layer is preferably thinner than a certain level, and the increase of the enzyme immobilization density relative to the projected area of the enzyme electrode is limited. On the other hand, in the method of physical adsorption of the enzyme on an above-mentioned conductive member composed of a carbonaceous material and a binder polymer, the conductive member has preferably a thickness not larger than a certain limit since the resistance between the carbonaceous material particles is high and this resistance increases with

the thickness of the enzyme-immobilizing layer containing the conductive member and the enzyme. Furthermore, in this method of using carbonaceous particles, a binder polymer is used for immobilizing the enzyme without using the carrier unlike in the entrapping immobilizing method, so that the enzyme-retaining ability is low and such type of enzyme electrode preferably is used for disposal type sensors. Therefore, this immobilization method is limited in improvement in electric charge transfer efficiency and expansion of the application fields.

The present invention intends to provide an enzyme electrode that can give a higher electric current density by increasing the enzyme immobilization density.

According to an aspect of the present invention, there is provided an enzyme electrode having a conductive member and an enzyme, wherein the conductive member has a porous structure, and the enzyme is immobilized through a carrier in pores constituting the porous structure.

The size of the pores on the surface side of porous structure of the conductive member is preferably larger than the size of the pores in the interior of the conductive member.

The enzyme electrode preferably contains a mediator for promoting transfer of electrons between

the enzyme and the conductive member.

The conductive member preferably comprises at least one of materials selected from metals, conductive polymers, metal oxides, and carbonaceous materials.

The enzyme is preferably a redox enzyme.

The conductive member preferably has at least two working faces opposing each other, and a liquid is permeable through the numerous voids between the two faces.

According to another aspect of the present invention, there is provided an enzyme electrode device, comprising the directly above-mentioned enzyme electrode, and wiring connected to the conductive member of the enzyme electrode.

In the enzyme electrode device, plural enzyme electrodes are preferably laminated with the working faces thereof opposed.

According to still another aspect of the present invention, there is provided a sensor, employing the enzyme electrode device as a detector for detecting a substance.

According to a further aspect of the present invention, there is provided a fuel cell having an anode and a cathode, and a region for retaining an electrolytic solution between the anode and cathode, wherein at least one of the anode and the cathode is

the enzyme electrode device.

According to a further aspect of the present invention, there is provided an electrochemical reactor having a reaction region, and an electrode for causing an electrochemical reaction of a source material introduced to the reaction region, wherein the electrode is the enzyme electrode device.

According to a further aspect of the present invention, there is provided a process for producing an enzyme electrode, comprising steps of:
providing a conductive member having numerous voids communicating with each other and communicating with the outside, and a carrier for immobilizing an enzyme for transfer of electrons to or from the conductive member; and
immobilizing the enzyme in the voids with immobilization of the carrier in the voids.

According to a further aspect of the present invention, there is provided a fuel cell, wherein an anode and a cathode have a porous structure, and at least one of the anode and the cathode is an enzyme electrode having an enzyme in pores constituting the porous structure.

The size of the pores on the surface side of the enzyme structure is preferably larger than the size of the pores in the interior of the enzyme electrode.

(Effect of the invention)

According to the present invention, an enzyme electrode can be provided which immobilizes an enzyme in a conductive member having numerous voids communicating with the outside of a conductive member having a large specific surface area at a high enzyme immobilization density by use of a carrier. In particular, in formation of a sheet-shaped or layered enzyme electrode, the electrode can be made thicker without increase of the interspace between the enzyme and the conductive member without lowering the electron transfer efficiency between the enzyme and the conductive member.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic drawing of an enzyme electrode immobilizing an enzyme by entrapping.

Fig. 2 is a schematic drawing of an enzyme electrode employing carbon particles as a member.

Fig. 3 is a schematic drawing of an enzyme electrode employing a conductive member having voids.

Fig. 4 shows a structure of a three-electrode cell.

Fig. 5A and Fig. 5B show dependence of an electric current density on a substrate concentration in a sensor.

Figs. 6A and 6B show dependence of an electric

current density on a substrate concentration in a sensor.

Fig. 7 shows a structure of a two-electrode cell.

Fig. 8 shows a structure of a five-layer flow cell.

Figs. 9A, 9B, 9C and 9D show examples of porous structure of the conductive members applicable in the present invention.

BEST MODE FOR CARRYING OUT THE PRESENT INVENTION

Preferred embodiments of the present invention are described below in detail.

An enzyme electrode of a preferred embodiment of the present invention comprises a conductive member having voids; and an enzyme for transferring electrons to or from the conductive member and a carrier for immobilizing the enzyme in the voids. This electrode is capable of immobilizing the enzyme on the conductive member stably by use of the carrier, and is capable of immobilizing the enzyme at a higher immobilization density for the effective surface area of the conductive member to improve the stability and the current density. This enzyme electrode has at least two working faces at the front side and the back side, and a liquid is permeable between the faces through numerous communicating voids in the

conductive member. For example, with a sheet-shaped (or film-shaped or layer-shaped) conductive member, openings of the voids are formed on the two faces (a front face and a back face) as the working face (contact face for contact with a liquid containing a component capable of interaction with the electrode), and the liquid is permeable from one operating face to the other operating face. The void openings may be formed also on a lateral side of the conductive member of the above shape to allow permeation of the liquid from the lateral face to the other face.

Further, the thickness of the enzyme electrode can be increased without increasing the distance between the enzyme and the conductive member and with little increase of the entire resistance of the electrode by use of a void-containing conductive member having a large effective area relative to its projected area, and high conductivity, for obtaining increased current density. Fig. 3 is a schematic drawing (a sectional view) of an enzyme electrode having a void-containing conductive member; and an enzyme for transferring electrons to or from the conductive member, and a carrier for immobilizing the enzyme in the voids. In the enzyme electrode of Fig. 3, enzyme 2 is immobilized by carrier 3 inside the voids of conductive member 8. The electric charge can be transferred, for example, as shown by arrow

mark 4. The voids in Fig. 3 communicate with the outside through other voids not shown in the drawing

The enzyme electrode connected with a wiring for electron transfer provides an enzyme electrode device useful for various application fields. This device employs the above enzyme electrode as a reaction electrode for an enzyme electrode reaction: the electrode may be constituted of a single layer or multiple layers of the above-mentioned sheet-shaped (or film-shaped or layer-shaped) enzyme electrode. In the plural enzyme electrode layers, the electrode layers may be arranged in lamination such that the front face of the one electrode layer confronts the reverse face of the other electrode layer. The multilayer electrode may be constituted of the same characteristic or may be constituted of a combination of enzyme electrodes of different characteristics. For example, similarly as a fuel cell mentioned later, the anodes and the cathodes are arranged alternately. This type of device can satisfy the requirement of the electric current, voltage, and output by changing the electrode structure from a monolayer structure to a multilayer structure. The enzyme, the catalyst constituting the enzyme electrode, has a high substrate selectivity in comparison with a noble metal catalyst (e.g., platinum) employed generally in electrochemical fields. Therefore, the reaction

substances on the anode and the cathode need not be separated by a partition, which can simplify the device. Further, the enzyme electrode employed in this device has continuous voids through the conductive member of the electrode. Therefore, an electrolyte solution can flow through the voids without providing an additional flow channel, whereby the device can be simplified. Further, a mechanism for promoting the penetration of the electrolyte solution provided outside the device can increase the supply of the substrate, whereby the electric current density can be increased.

A sensor, a preferred embodiment of the present invention, employs a device having a monolayer or multilayer enzyme electrode as a detector portion for detecting a substance. In a typical constitution of the sensor, an enzyme electrode is employed as the working electrode in combination with a counter electrode, and with a reference electrode if necessary, whereby an electric current is detected by the enzyme electrode (by the function of the enzyme immobilized on the enzyme electrode) to detect a substance in a solution in contact with the electrodes. The constitution of the sensor is not limited insofar as the enzyme electrode is capable of the detection. Fig. 4 shows an example of the sensor. In Fig. 4, the sensor comprises anode 12, platinum

wire electrode 13, and silver-silver chloride reference electrode 14. The respective electrodes are connected by leading wires 15,16,20 to potentiostat 18. This sensor is placed in electrolyte solution 11 in water-jacketed cell 9 tightly closable with cover 10. A substrate in the electrolyte can be detected by applying a potential to the working electrodes and measuring the steady-state current. When the measurement should be conducted in an inert atmosphere, an inert gas like nitrogen is introduced from gas inlet 19 of gas tube 20. The temperature of the measurement solution can be controlled by feeding a temperature-controlling liquid from temperature controlling liquid inlet 21 to temperature controlling liquid outlet 22. This sensor has high substrate selectivity owing to the enzyme employed as the electrode reaction catalyst, and achieves a high current density owing to the enzyme electrode employing a void-containing conductive member, whereby the detection reactor can be simplified, or the detector portion can be miniaturized. This sensor is capable of detecting a substance corresponding to the substrate of the enzyme of the enzyme electrode, being useful, for example, as a glucose sensor, a fructose sensor, a galactose sensor, an amino acid sensor, an amine sensor, a cholesterol sensor, an alcohol sensor, a

lactic acid sensor, an oxygen sensor, a hydrogen peroxide sensor, or the like. More specific application examples are a sensor for measuring a glucose concentration or lactic acid concentration in blood, a sensor for measuring a sugar concentration in a fruit, and a sensor for measuring an alcohol concentration in exhaled breath.

A fuel cell, another preferred embodiment of the present invention, employs a device having a monolayer or multilayer enzyme electrode as at least one of the anode and cathode thereof. In the multilayer constitution, the anodes and the cathodes may be placed in a predetermined arrangement in the lamination direction. A typical constitution of the fuel cell has a reaction vessel for holding an electrolyte solution containing a fuel material, and the anode and the cathode placed at a predetermined spacing in the reaction vessel, at least one of the anode and the cathode employing an enzyme electrode of the present invention. The fuel cell may be of a type in which an electrolyte solution is replenished or circulated, or may be of a type in which an electrolyte solution is neither replenished nor circulated. The fuel cell is not limited in the fuel, the structure, the function, and so forth, insofar as the enzyme electrode is usable. This fuel cell can give a high driving voltage by redox of a substance

at a low overvoltage owing to a characteristic high activity of the employed enzyme as the catalyst for the electrode reaction. The fuel cell can give also a high electric current density by using an enzyme electrode employing a void-containing conductive member, whereby a high output and/or miniaturization of the fuel cell can be realized. Fig. 8 shows an example of the fuel cell. The fuel cell shown in Fig. 8 has an electrode unit having anodes 12 connected to anode lead wires 15 and cathodes 24 connected to cathode lead wires laminated with interposition of porous polypropylene films 23, encased in acrylic case 27. An electrolyte solution is introduced from electrolyte solution inlet 25 and is discharged from electrolyte solution outlet 26 to function as a fuel cell.

An electrochemical reactor, still another embodiment of the present invention, employs a device having a monolayer or multilayer enzyme electrode as the reaction electrode. Typically, the reactor has a pair of electrodes and optionally a reference electrode. The electrodes are placed in a reaction vessel for holding a reaction solution, and an electric current is allowed to flow between the pair of electrodes to cause an electrochemical reaction of a substance in the reaction solution to obtain an intended reaction product, a decomposition product,

or the like. At least one of the pair of the electrodes is an enzyme electrode of the present invention. The kind of the reaction solution, the reaction conditions, and the constitution of the reactors are not specially limited, insofar as the enzyme electrode is usable. For example, the reactor is useful for preparation of a redox reaction product, or a decomposition product. Fig. 4 and Fig. 7 show specific examples of the constitution of the reactor. The reactor shown in Figs. 4 or 7 as the electrochemical reactor produces an intended product by application of an electric current or a voltage to cause electrochemical reaction in contrast to the aforementioned sensor or fuel cell.

The electrochemical reactor can achieve quantitativeness of the electrochemical reaction as well as high selectivity and high catalytic activity specific to the enzyme employed as the electrode reaction catalyst. Therefore, a reactor can be produced which can be operated with high selectivity, high efficiency, and high quantitativeness. This electrochemical reactor can cause selectively the reaction of a substance corresponding to the substrate of the enzyme of the enzyme electrode, being useful for oxidation of glucose, fructose, galactose, amino acids, amines, cholesterol, alcohols, lactic acid, and so forth; reduction of oxygen,

hydrogen peroxide, and so forth; and the like reactions. More specific application examples include selective oxidation of cholesterol in the presence of ethanol, and reduction of oxygen at a low overvoltage.

The numerous voids in the conductive member are interconnected together in one-, two-, or three-dimensionally. The interconnection of the voids may be of two or more types. The one-dimensional void interconnection is exemplified by columnar voids; the two-dimensional void interconnection is exemplified by net-like voids; and the three-dimensional void interconnection is exemplified by sponge-like void, interstices formed in aggregation of small particles, and voids in a structural material prepared by use of the above material as a template. The voids should be large for introduction of the enzyme and flow and diffusion of the substrate substance, but should be small within the range for obtaining a sufficient ratio of the effective void surface area to the projected area of the member. The average void diameter ranges, for example, from 5 nm to 5 mm, more preferably from 10 nm to 500 μm. The conductive member should have a small thickness for flow and diffusion of the substrate through the member, but should have thickness within the range for obtaining a sufficient ratio of the effective void surface area

to the projection area of the conductive member. The thickness of the void-containing conductive member ranges, for example, from 100 nm to 1 cm, more preferably from 1 μm to 5 mm. The ratio of the effective surface area to the projected area of the void-containing conductive member should be sufficiently large, for example, the ratio being 10 or more, more preferably 100 or more. The porosity of the void-containing conductive member should be sufficiently large for obtaining a high ratio of the effective void surface area to the projected area of the conductive member, and be large within the range for enabling introduction of the enzyme and the carrier and flow and diffusion of the substrate substance, but should not be excessively large for achieving the sufficient mechanical strength. The porosity ranges, for example, from 20% to 99%, more preferably from 30% to 98%. The porosity of the conductive member having an enzyme immobilized therein should be large for flow of the electrolyte solution and diffusion of the substrate substance, but should be small by filling of the enzyme. The porosity ranges for example, from 15% to 98%, more preferably from 25% to 95%.

The voids may be narrowed toward the inside from the surface of the conductive member in contact with the electrolyte solution, namely the outside

surface having opening communicating with the inside voids of the electroconductive member. This type of conductive member is hereinafter referred to as a void size (e.g., pore size)-gradient conductive member having numerous voids. For holding at a high density the enzyme effective to the electrode reaction, it is effective to use conductive member having numerous voids smaller than a certain size. However, with the enzyme held at a high density, diffusion of the substrate substance to the enzyme can restrict the total electric current flow of the entire electrode, and sufficient diffusion of the substrate substance into the interior of the void-containing conductive member may not be achieved. To offset the disadvantage, use of the void size-gradient conductive member having numerous voids enables the sufficient holding density of the enzyme effective to the electrode reaction as well as sufficient diffusion of the substrate substance into the interior of the conductive member. The void size-gradient conductive member having numerous voids may be produced initially to have the void size gradient, or may be prepared by laminating conductive members having pores of different sizes. Otherwise, the member may be prepared by laminating plural members having different component compositions. The average void diameter of the void size-gradient

conductive member having numerous voids ranges, for example, from 100 nm to 5 mm, more preferably from 1 μm to 1 mm in the larger void portion, and ranges from 5 nm to 500 μm in the smaller void portion. The void-size gradient region in a plate-shaped conductive member, for example, may be formed such that the size of the voids changes continuously or stepwise from one of the opposing face (front face) toward the other face (back face): in other words, voids at the back face side are smaller in size than the voids at the front face side. Otherwise, the voids may be formed to be smaller gradually from front face and the back face toward the center. The void-size gradient may be decided to meet the intended uses.

In the conductive member for the enzyme electrode of the present invention, naturally the voids may have a uniform size (or uniform porosity) in the thickness direction of the porous structure, or the voids may have a gradient distribution of the size (or porosity).

Figs. 9A, 9B, 9C and 9D illustrates porous structures of the conductive member. In the drawings, the numerals denote the followings: 801, an electrolyte layer; 802, a pore; 803, a conductive member; 804, supporting substrate optionally employed. As shown in the drawings, the sizes of the pores in

the conductive member are preferably larger at the electrolyte layer side (i.e., outer surface side of the conductive member) and smaller in the inside (i.e., interior of the conductive member). In other words, in the conductive porous member employed in the present invention, the pore sizes are preferably larger at the surface side of the conductive porous member than those at the interior thereof. The pore size ratio is preferably 2 or more, more preferably 4 or more, still more preferably 10 or more, but is not larger than 1000.

The porosity may be the same between the regions of different pore sizes. More preferably, the pore sizes and the porosities in the conductive member are both larger in the electrolyte layer side, and smaller in the interior. The pore size and porosity of the porous member can be measured by nitrogen gas adsorption measurement (BET method (Brunauer-Emmett-Teller method)), for example by AUTOSORB-1 (Quantachrome Instruments Co.). The pore sizes on the surface of the member can be estimated by measuring the pore sizes of a certain number of pores (e.g., 50 to 300 pores) in SEM photograph (scanning electron microscope photograph).

The conductive porous layer constituting the enzyme electrode has preferably a region in which the pore size is decreased from the electrolyte side of

the porous layer toward the other face side. The size of the pores in the porous layer of the present invention may be changed, from one face side (electrolyte side) toward the other face side, to have a high-porosity region, and a low-porosity region; or a high-porosity region, a medium-porosity region, and a low-porosity region; or a high-porosity region, a low-porosity region, and a high-porosity region.

The carrier serves at least to immobilize the enzyme to the conductive member. The carrier includes (1) polymer compounds, (2) inorganic compounds, and (3) organic compounds, the compounds having a covalent bonding site in the molecule and being capable of bonding an enzyme to the conductive member, and/or the two enzymes. The carrier contains at least one of the above three types of compounds. To immobilize the enzyme to the electrode, the carrier has preferably an electric charge opposite to the surface charge of the enzyme under the electrode driving conditions. The carrier may be ones capable of holding the enzyme by covalent bonding, electrostatic interaction, spatial trapping, or a like action to hold the enzyme stably at a high density in comparison with retention of the enzyme by physical adsorption to the electrode or to a binder polymer for caking the electrode.

The polymer compounds useful as the carrier include electroconductive polymers such as polyacetylenes, polyarylenes, polyarylene-vinylenes, polyacenes, polyarylacetylenes, polydiacetylenes, polynaphthalenes, polypyrroles, polyanilines, polythiophenes, polythienylenes, vinylenes, polyazulenes, and polyisothianaphthenes; and other kind of polymers such as polystyrenesullfonic acids, polyvinyl sulfate, dextran sulfate, chondroitin sulfate, polyacrylic acid, polymethacrylic acid, polymaleic acid, polyfumaric acid, polyethylenimine, polyallylamine hydrochloride, polydiallyldimethylammonium chloride, polyvinylpyridine, polyvinylimidazole, polylysine, deoxyribonucleic acid, ribonucleic acid, pectin, silicone resins, cellulose, agarose, dextran, chitin, polystyrene, polyvinyl alcohol, and nylons.

The inorganic compounds useful as the carrier include metal chalcogenide compounds containing at least one element selected from the group of In, Sn, Zn, Ti, Al, Si, Zr, Nb, Mg, Ba, Mo, W, V, and Sr.

The organic compounds, being useful as the carrier, and having a covalent bonding site in the molecule and being capable of bonding an enzyme to the conductive member, and/or the two enzymes include compounds having at least one functional group selected from hydroxyl, carboxyl, amino, aldehydo,

hydrazino, thiocyanato, epoxy, vinyl, halogeno, acid ester groups, phosphat0, thiol, disulfido, dithiocarbamato, dithiophosphato, dithiophosphnato, thioether groups, thiosulfato, and thiourea groups. Typical examples are glutaraldehyde, polyethylene glycol diglycidyl ether, cyanuric chloride, N-hydroxysuccinimide esters, dimethyl-3,3'-dithiopropionimidate hydrochloride, 3,3'-dithio-bis(sulfosuccinimidyl propionate), cystmine, alkyl dithiols, biphenylene dithiols, and benzene dithiols.

The mediator serves to promote transfer of electrons between the enzyme and the conductive member, and may be employed optionally as necessary. The mediator may be chemically bonded to at least one of the carrier and the enzyme. The mediator is exemplified by metal complexes, quinones, heterocyclic compounds, nicotinamide derivatives, flavin derivatives, electroconductive polymers, electroconductive fine particulate materials, and carbonaceous materials. The metal complexes include those having as the central metal at least one element selected from Os, Fe, Ru, Co, Cu, Ni, V, Mo, Cr, Mn, Pt, Rh, Pd, Mg, Ca, Sr, Ba, Ti, Ir, Zn, Cd, Hg, and W. The ligands of the metal complexes are exemplified by those containing an atom of nitrogen, oxygen, phosphorus, sulfur, or carbon and capable of forming a complex through the above atom with the

central metal; and those having a cyclopentadienyl ring as the skeleton. The ligand includes pyrrole, pyrazole, imidazole, 1,2,3- or 1,2,4-triazole, tetrazole, 2,2'-biimidazole, pyridine, 2,2'-bithiophene, 2,2'-bipyridine, 2,2':6'2"-terpyridine, ethylenediamine, porphyrin, phthalocyanine, acetylacetone, quinolinol, ammonia, cyan ion, triphenylphosphine oxide, and derivatives thereof. The quinines as the mediator include quinone, benzoquinone, anthraquinone, naphthoquinone, pyrroloquinolinequinone, tetracyanoquinodimethane, and derivatives thereof. The heterocyclic compounds as the mediator include phenazine, phenothiazine, biologen, and derivatives thereof. The nicotinamide derivatives as the mediator include nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide phosphate. The flavin derivatives as the mediator include flavin adenine dinucleotide (FAD). The electroconductive polymers as the mediator include polyacetylenes, polyarylenes, polyarylene-vinylenes, polyacenes, polyarylacetylenes, polydiacetylenes, polynaphthalenes, polypyrroles, polyanilines, polythiophenes, polythienylenevinylenes, polyazulenes, and polyisothianaphthenes. The electroconductive fine particulate materials as the mediator contain a fine particulate metal material including metals containing at least one element of

Au, Pt, Ag, Co, Pd, Rh, Ir, Ru, Os, Re, Ni, Cr, Fe, Mo, Ti, Al, Cu, V, Nb, Zr, Sn, In, Ga, Mg, and Pb; and fine particulate electroconductive polymers: the material may be an alloy or may be plated. The carbonaceous materials as the mediator include fine particulate graphite, fine particulate carbon black, fullerene compounds, carbon nanotubes, carbon nanohorns, and derivatives thereof.

The conductive member has numerous voids formed inside and communicating with the outside: preferably partitions are formed from the constituting material in integration to separate the voids, or partitions separating the voids are tightly bonded. The constituting material of the conductive member includes electroconductive materials such as metals, polymers, metal oxides, and carbonaceous materials.

The metal for constituting the conductive member should have electroconductivity, sufficient rigidity during storage and measurement operation, and sufficient electrochemical stability under the electrode working conditions. The metal includes those containing at least one element of Au, Pt, Ag, Co, Pd, Rh, Ir, Ru, Os, Re, Ni, Cr, Fe, Mo, Ti, Al, Cu, V, Nb, Zr, Sn, In, Ga, Mg, Pb, Si, and W. The metal may be an alloy, or a metal-plated matter. The void-containing metal includes foamed metals, electrodeposited metals, electrolytic metals,

sintered metals, fibrous metals, and metals corresponding to two or more of the above kinds of metals. The electric conductivity of the conductive member applicable to the present invention ranges from 0.1 to 700000 S/cm, preferably from 1 to 100000 S/cm, more preferably from 100 to 100000 S/cm. (Incidentally, S denotes siemens, a reciprocal of ohm (1/Ω).) The conductive member having the porous structure for the enzyme electrode has preferably the electric conductivity within the above range.

The electroconductive polymer for constituting the conductive member should have electroconductivity, sufficient rigidity during storage and measurement operation, and sufficient electrochemical stability under the electrode working conditions. The polymer includes those containing at least one compound selected from polyacetylenes, polyarylenes, polyarylene-vinylenes, polyacenes, polyarylacetylenes, polydiacetylenes, polynaphthalenes, polypyrroles, polyanilines, polythiophenes, polythienylenevinylenes, polyazulenes, and polyisothianaphthenes. This void-containing polymer can be produced by any of the processes for manufacture of a porous resin. In one process, a template for the voids is used in molding a conductive polymer into an intended shape, and thereafter the material of the template is removed. In another process, a template for the voids is

placed in a prepolymer, the prepolymer is polymerized into a conductive polymer, and thereafter the material of the template is removed. In a still another process, a layer is formed from particles for constituting a void template, a polymer is filled into the interstice of the particle layer, and thereafter the particles are removed from the layer. In a still another process, a layer is formed from particles for constituting a void template, a prepolymer is filled into the interstice of the particle layer, the prepolymer is polymerized to form a polymer layer, and thereafter the particles are removed from the layer.

The metal oxide for constituting the conductive member should have sufficient rigidity during storage and measurement operation, and sufficient electrochemical stability under the electrode working conditions. The metal oxide may be improved in electroconductivity or may be made electroconductive by an additional electroconductive material. The metal oxide includes those containing at least one element of In, Sn, Zn, Ti, Al, Si, Zr, Nb, Mg, Ba, Mo, W, V, and Sr. The additional electroconductive material includes metals, electroconductive polymers, and carbonaceous materials. The metal oxide production process includes electrodepositing, sputtring, sintering, chemical vapor deposition (CVD),

electrolysis, and combination thereof.

The carbonaceous material for constituting the conductive member in the present invention should have sufficient rigidity during storage and measurement operation, and sufficient electrochemical stability under the electrode working conditions. The carbonaceous material may be improved in electroconductivity or may be made electroconductive by an additional electroconductive material. The carbonaceous material includes graphite, carbon black, carbon nanotubes, carbon nanohorns, fullerene compounds, and derivatives thereof. The conductive member can be produced from the carbonaceous material by sintering.

As the enzyme to be immobilized on the conductive member, oxidoreductases are useful. The oxidoreductase catalyzes a redox reaction. Plural different enzymes may be combinedly immobilized on one and the same enzyme electrode for achieving an intended characteristic. The enzymes include glucose oxidase, galactose oxidase, bilirubin oxidase, pyruvate oxidase, D- or L-amino acid oxidase, amine oxidase, cholesterol oxidase, choline oxidase, xanthine oxidase, sarcosine oxidase, L-lactate oxidase, ascorbate oxidase, cytochrome oxidase, alcohol dehydrogenase, glutamate dehydrogenase, cholesterol dehydrogenase, aldehyde dehydrogenase,

glucose dehydrogenase, fructose dehydrogenase, sorbitol dehydrogenase, lactate dehydrogenase, maleate acid dehydrogenase, glycerol dehydrogenase, 17B-hydroxysteroid dehydrogenase, estradiol-17B dehydrogenase, amino acid dehydrogenase, glyceraldehyde-3-phoshate dehydrogenase, 3-hydroxysteroid dehydrogenase, diaphorase, cytochrome C catalase, peroxidase, glutathione reductase, NADH-cytochrome b5 reductase, NADPH-adrenodoxin reductase, cytochrome b5 reductase, adrenodoxin reductase, and nitrate reductase.

The substrate substances for the enzymes are compounds corresponding to the respective enzymes, including organic matters, oxygen, hydrogen peroxide, water, and nitrate ions. The organic matters include sugars, alcohols, carboxylic acids, quinones, nicotinamide derivatives, and flavin derivatives. The sugars include polysaccharides such as cellulose, and starch.

In the carrier immobilization in the present invention, the carrier is preferably uniformly immobilized in the voids of the conductive member. For the uniform immobilization of the carrier in the voids of the conductive member, the surface of the conductive member is preferably made hydrophilic prior to introduction of the carrier into the conductive member. The process for hydrophilicity

treatment of the surface of the conductive member includes UV-ozone treatment; permeation of a water-soluble organic solvent like an alcohol into the voids of the conductive member and substitution of the solvent with water; and application of ultrasonic wave during the above hydrophilicity treatment. The carrier immobilization process may be conducted simultaneously with the enzyme immobilization process and/or mediator immobilization process. The immobilization of the carrier may be conducted, for example, by any of the processes below. In a process, a void-containing conductive member is immersed in a solution or dispersion of the carrier. In another process, a solution or dispersion of the carrier is applied, injected, or sprayed to the void-containing member. In still another process, a void-containing conductive member is immersed in a solution or dispersion of the carrier precursor, or a solution or dispersion of the carrier precursor is applied, injected, or sprayed to the void-containing member, and the carrier precursor is hydrolyzed, polymerized, or crosslinked for immobilization.

(Examples)

The present invention is explained below in more detail without limiting the invention thereto.

Firstly, a method of preparation of the void-containing conductive member used in the present

invention is described. The size of the particles can be measured by scanning electron microscopy. The film thickness can be measured by surface roughness tester.

(Preparation Example 1)

A commercial polystyrene type latex colloid dispersion liquid (Nippon Zeon Co.; average particle size: 100 nm) is employed. The dispersion medium of the dispersion liquid is replaced by ethanol. A cleaned gold substrate is allowed to stand in the dispersion liquid. The ethanol is allowed to evaporate at 30°C to obtain a porous film constituted of polystyrene spheres. This process is repeated several times to obtain a porous film constituted of polystyrene spheres of an intended film thickness (100 μm thick). The film is heated at 70°C for 30 minutes, and then washed with ethanol. Using this porous film as the working electrode and a platinum electrode as the counter electrode, electro-deposition is conducted in an aqueous 0.1M nickel sulfate solution at a current density of 0.1 mA/cm^2 by control with a galvanostat. The time of the electro-deposition is controlled by monitoring the electrolysis current profile to obtain a film in a thickness nearly equivalent to the polystyrene film thickness. After the electro-deposition, the film is immersed in toluene for two days to remove the latex

to obtain a conductive member constituted of nickel having numerous voids.

(Preparation Example 2)

A platinum paste (Tanaka Kikinzoku Kogyo K.K.; platinum particle size: 1 μm) is applied on a cleaned gold substrate by screen process printing, and is sintered at 500°C for one hour to obtain a conductive member (100 μm thick) constituted of platinum having numerous voids.

(Preparation Example 3)

A gold paste (Tanaka Kikinzoku Kogyo K.K.; gold particle size: 1 μm) is applied on a cleaned gold substrate by screen process printing, and is sintered at 500°C for one hour to obtain a conductive member (100 μm thick) constituted of gold having numerous voids.

(Preparation Example 4)

Palladium particles (Tanaka Kikinzoku Kogyo K.K.; particle size: 1 μm) is dispersed in an about double weight of terpinol, and the viscosity is adjusted by addition of ethylcellulose to obtain a palladium paste. This palladium paste is applied on a cleaned gold substrate by screen process printing, and is sintered at 500°C for one hour to obtain a conductive member (100 μm thick) constituted of palladium having numerous voids.

(Preparation Example 5)

A commercial silica colloid dispersion liquid (Nissan Chemical Ind.; average particle size: 100 nm) is employed. The dispersion medium of the dispersion liquid is replaced by ethanol. A cleaned gold substrate is allowed to stand in the dispersion liquid. The ethanol is allowed to evaporate at 30°C to obtain a porous film constituted of silica spheres. This process is repeated several times to increase the thickness of a porous film constituted of silica spheres (100 nm thick). The film is heated at 200°C for three hours, and then washed with ethanol. In a three-electrode cell, by use of this porous film as the working electrode, a platinum electrode as the counter electrode, and an Ag/AgCl electrode as the reference electrode, electrolytic polymerization is conducted in a solution of 0.1M pyrrole and 0.1M lithium perchlorate in acetonitrile at a potential of 1.1 V (vs Ag/AgCl) by means of a potentiostat. The time of the polymerization is controlled by monitoring the electrolysis current profile to obtain a film in a thickness nearly equivalent to the silica sphere porous film thickness. After the electrolytic polymerization, the film is immersed in a 20% hydrofluoric acid solution for two days to remove the silica spheres to obtain a conductive member (100 μm thick) constituted of electroconductive polypyrrole containing numerous voids.

(Preparation Example 6)

A conductive member (100 μm thick) composed of poly(3,4-ethylenedioxythiophene) having numerous voids is prepared in the same manner as in Preparation Example 5 except that 3,4-ethyenedioxythiophene is used instead of pyrrole.

(Preparation Example 7)

A commercial aqueous dispersion of poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (Bayer) is used. The dispersion medium of this dispersion is replaced by ethanol (polymer concentration: 10 g/L). This solution is dropped onto a porous film constituted of silica spheres prepared in the same manner as in Preparation Example 5, and dried. This process is repeated to fill the polymer in the voids of the silica-sphere porous film. Then the film is annealed at 70°C for 30 minutes. Further, the film is immersed in a 20% hydrofluoric acid solution for two days to remove the silica spheres to obtain a conductive member constituted of poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) having numerous voids.

(Preparation Example 8)

A porous film constituted of silica spheres is prepared in the same manner as in Preparation Example 5. By use of this porous film as the working electrode and a platinum wire as the counter

electrode, electro-deposition is conducted in an aqueous solution of 0.5M aniline and 1M lithium perchlorate at a current density of 0.1 mA/cm^2 by control with a galvanostat. The time of the electro-deposition is controlled by monitoring the electrolysis current profile to obtain a film in a thickness nearly equivalent to the porous silica sphere film thickness. After the electro-deposition, the film is immersed in a 20% hydrofluoric acid solution for two days to remove the silica spheres to obtain a conductive member constituted of polyaniline containing numerous voids.

(Preparation Example 9)

Needle-shaped indium tin oxide (ITO, Sumitomo Metal Mining Co.; length: 30-100 nm; aspect ratio: 10 or higher) is dispersed in terpinol, and the viscosity is adjusted by addition of ethylcellulose to obtain an ITO paste. This ITO paste is applied on a cleaned gold substrate by screen process printing, and is sintered at 250°C for one hour to obtain a porous ITO sintered electrode (100 μm thick). Further thereon, ITO is deposited by plasma chemical vapor phase deposition (CVD) in a thickness of about 10 nm to obtain a conductive member constituted of ITO and having numerous voids.

(Preparation Example 10)

Commercial fine particulate electroconductive

titanium oxide (Titan Kogyo K.K.; EC-300; particle diameter: 300 nm) is dispersed in terpinol, and the viscosity is adjusted by addition of ethylcellulose to obtain a titanium oxide paste. This titanium oxide paste is applied on a cleaned gold substrate by screen process printing, and is sintered at 450°C for one hour to obtain a sintered porous titanium oxide film (100 μm thick). By use of this porous film as the working electrode and a platinum wire as the counter electrode, electrolytic plating is conducted in a gold-plating solution (Kamimura Kogyo K.K.; 535LC) with an ultrasonic vibration at a current density of 0.1 mA/cm^2 by control with a galvanostat for one hour, blowing a jet of the gold-plating solution on the sintered porous film, to obtain a conductive member constituted of gold-plated porous titanium oxide having numerous voids.

(Preparation Example 11)

A cleaned gold substrate is immersed in a 0.01M zinc nitrate solution in water/ethanol (9:1). On this base plate, needle-shaped zinc crystal is allowed to grow by application of a potential of -1.2 V (vs Ag/AgCl) by employing a platinum wire as the counter electrode and an Ag/AgCl electrode as the reference electrode at 85°C for 1.5 hours. After washing the base plate, the crystalline matter is treated for coating with carbon as below. The base

plate is placed in a tubular furnace. The temperature is elevated by 5°C per minute to the predetermined temperature. During the heat treatment, hydrogen/helium (2%/98%) is constantly fed at a flow rate of 33 sccm. During the thermal decomposition of hydrocarbon, ethylene/helium (1%/99%) is fed at 66 sccm as a hydrocarbon gas. During the thermal decomposition of the hydrocarbon, the total gas feed rate is 100 sccm at the gas ratio of ethylene:hydrogen:helium = 1:1:100. In the heat treatment, the temperature is elevated in an atmosphere of hydrogen/helium (2%/98%) up to 1000°C in 200 minutes, the temperature is kept for 10 minutes, and then ethylene/helium (1%/99%) is fed for 10 minutes. The system is kept at 1000°C for 1 hour, and cooled in 200 minutes. Thereby a conductive member is prepared which has numerous voids constituted of carbon-coated needle-shaped crystalline zinc oxide.

(Preparation Example 12)

An electropolished aluminum sheet (100 μm thick) is anodized in 0.3M sulfuric acid at 25 V for one hour to obtain porous alumina at pore intervals of 60 nm. This porous alumina sheet is electroplated with a platinum counter electrode in a gold electroplating solution (Kamimura Kogyo K.K.; 535LC) in a jet flow of said gold-plating solution with an

ultrasonic vibration at a current density of 0.1 mA/cm^2 by control with a galvanostat for one hour. Thereby a conductive member is prepared which is constituted of porous alumina having many gold-plated pores.

(Preparation Example 13)

Natural particulate graphite (particle size: 11 μm) is mixed with polyvinylidene fluoride in an amount of 10 wt% of the particulate graphite. N-methyl-2-pyrrolidone is added thereto to solve the polyvinylidene fluoride. The blended graphite paste is molded into a film of 11.3 mm diameter and 0.5 mm thick. The film is dried at 60°C, heated to 240°C, and further vacuum-dried at 200°C. Thereby a conductive member is obtained which is constituted of many graphite particles bonded together and has numerous voids in the structure.

(Preparation Example 14)

A conductive member is prepared in the same manner as in Preparation Example 13 except that carbon black (Lion Corp.; Carbon ECP600JD) is used instead of the particulate graphite. Thereby a conductive member is obtained which has numerous voids in the carbon black particle structure.

(Preparation Example 15)

A conductive member is prepared in the same manner as in Preparation Example 14 except that monolayer carbon nanotubes (Carbon Nanotech Research

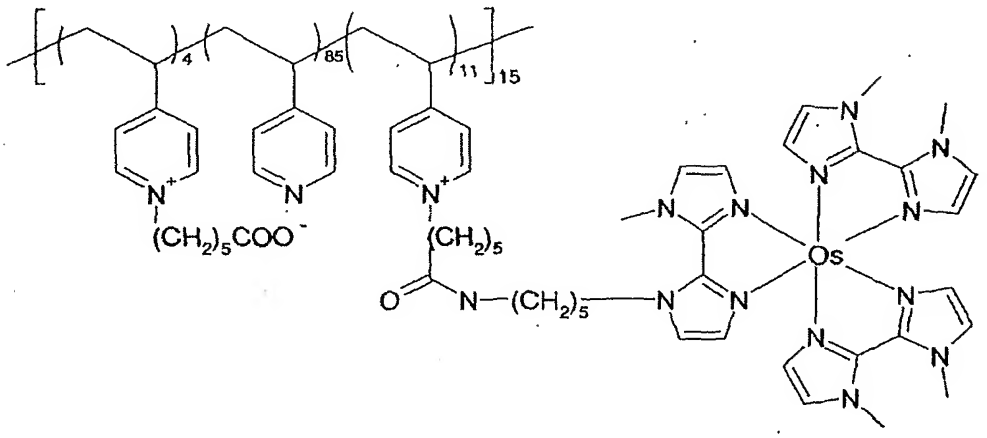
Institute) is used in an amount of 20 wt% of the carbon black. Thereby a conductive member is obtained which has numerous voids in the carbon nanotube structure.

Next, processes for preparation of the mediator are described below.

(Preparation Example 16)

The process for synthesis of the complex polymer shown by Chemical Formula (1) is described below.

(Chemical Formula 1)



....(1)

To 100 g of an aqueous 40% glyoxal solution, was added dropwise 370 mL of an aqueous concentrated ammonia solution on an ice bath. The mixture is stirred at 45°C for 24 hours, and is air-cooled. The formed precipitate is collected by filtration, and vacuum-dried at 50°C for 24 hours to obtain 2,2'-

biimidazole. This compound is identified by silica-gel thin-layer chromatography (methanol/chloroform (10%/90%)). To a solution of 4.6 g of 2,2'-biimidazole in 100 mL of N,N'-dimethylformamide (DMF), is added 2.7 g of sodium hydride in a nitrogen atmosphere on an ice bath. The mixture is stirred at room temperature for one hour. Thereto, a solution of 12.8 g of methyl p-toluenesulfonate in 5 mL of DMF is added dropwise in 20 minutes, and the mixture is stirred at room temperature for 4 hours. The solvent is evaporated under vacuum at 50°C. The evaporation residue is washed with 50 mL of hexane, and vacuum-dried at 160°C to obtain N,N'-dimethyl-2,2'-biimidazole in a colorless transparent crystalline state. The obtained product is identified by ^{1}H-NMR.

To a solution of 10 g 2,2'-biimidazole in 100 mL of DMF, is added 3.3 g of sodium hydride on an ice bath in a nitrogen atmosphere. The mixture was stirred on an ice bath for one hour. Thereto, 4.6 mL of methyl iodide is added dropwise, and the mixture was stirred on an ice bath for 30 minutes and at room temperature for 12 hours. The reaction solution is poured into 300 mL of ethyl acetate. The mixture is filtered, and the solvent is evaporated from the filtrate under a reduced pressure and vacuum. The evaporation residue is dissolved in boiling ethyl acetate, and the solution is filtered. The filtered

ethyl acetate solution is boiled again. Thereto 300 mL of hexane is added for saturation. The solution is kept in a refrigerator for 12 hours for crystal growth. The crystalline matter is collected by suction filtration, and recrystallized from ethyl acetate/hexane to obtain N-methyl-2,2'-biimidazole. The identification is conducted by ^{1}H-NMR.

A 1 g portion of N-methyl-2,2'-biimidazole is dissolved in 80 mL of DMF. Thereto, 0.32 g of sodium hydride is added in a nitrogen atmosphere. The mixture is stirred on an ice bath for one hour. Thereto 2.5 g of N-(6-bromohexyl)phthalimide and 1.0 g of sodium iodide are added gradually. The mixture is stirred in a nitrogen atmosphere at 80°C for 24 hours. The mixture is cooled to room temperature, and 150 mL of water is added thereto. The mixture is extracted twice with ethyl acetate. The ethyl acetate solution is washed with an aqueous sodium chloride solution and dried over sodium sulfate, and is evaporated under a reduced pressure. The residue is purified by a neutral alumina column (ethyl acetate/hexane 10 to 40%) to obtain N-methyl-N'-(6-phthalimidohexyl)-2,2'-biimidazole. This product is identified by ^{1}H-NMR.

A 2.5 g portion of N-methyl-N'-(6-phthalimidohexyl)-2,2'-biimidazole is dissolved in 25 mL of ethanol, and thereto 0.39 g of hydrogenated

hydrazine is added. The mixture is refluxed for 2 hours, cooled to room temperature, and filtered. The solution is transferred to a silica gel column with ethanol. The product is recovered by a 10% ammonia solution in acetonitrile, and the solution is evaporated under a reduced pressure to obtain N-(6-aminohexyl)-N'-methyl-2,2'-biimidazole. This product is identified by ^{1}H-NMR.

In 40 mL of ethylene glycol, 1.1 g of N-methyl-2,2'-biimidazole and 1.4 g of ammonium hexachloroosmate are dissolved. The solution is stirred in a nitrogen atmosphere at 140°C for 24 hours. Thereto, is added a solution of 0.8 g of N-(6-aminohexyl)-N'-methyl-2,2'-biimidazole in 5 mL of ethylene glycol. The solution is stirred further for 24 hours, cooled to room temperature, and filtered. The filtrate is diluted with 200 mL of water, and stirred with 40 mL of an anion exchange resin (DOWEX® 1X4) in the air for 24 hours. The solution is poured gradually into a solution of 10.2 g of ammonium hexafluorophosphate in 150 mL of water. The precipitate is collected by filtration by suction, and dissolved in acetonitrile and reprecipitated by an aqueous ammonium hexafluorophosphate solution. The obtained matter is washed with water, and vacuum-dried at 45°C for 24 hours to obtain osmium(III) (N,N'-dimethyl-2,2'-biimidazole)2(N-(6-aminohexyl)-

N'-methyl-2,2'-biimidazole) hexafluorophosphate salt. This product is identified by elemental analysis.

To 150 mL of DMF, are added 20 g of polyvinylpyridine (average molecular weight: 150,000) and 5.6 g of 6-bromohexane. The mixture is stirred at 90°C with a stirrer for 24 hours, and cooled to room temperature. The cooled mixture is poured gradually into 1.2 L of ethyl acetate with violent agitation. Then the solvent is removed by decantation, and the remaining solid matter is dissolved in methanol. The solution is filtered, and evaporated to a solvent volume of about 200 mL. The formed product is reprecipitated with 1 L of diethyl ether. The product is vacuum-dried at 50°C for 24 hours, pulverized, and further dried for 48 hours to obtain poly(4-(N-(5-carboxypentyl)pyridinium)-co-4-vinylpyridine).

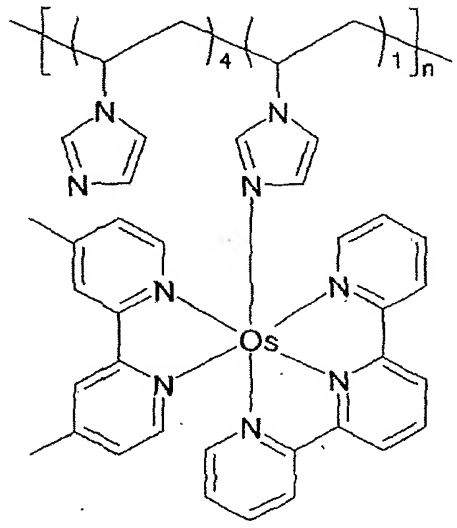
In 10 mL of DMF, 0.52 g of the poly(4-(N-(5-carboxypentyl)pyridinium)-co-4-vinylpyridine) is dispersed, and thereto 0.18 g of O-(N-succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TSTU) is added. The mixture is stirred for 15 minutes. Thereto 0.1 mL of N,N-diisopropylethylamine is added, and the mixture is stirred for 8 hours. Thereto, 0.89 g of poly(4-(N-(5-carboxypentyl)pyridinium)-co-4-vinylpyridine) is added and the mixture is stirred for 5 minutes. Further thereto, 0.1 mL of N,N-

diisopropylethylamine is added and the mixture is stirred at room temperature for 24 hours. The resulting mixture is added to 200 mL of ethyl acetate. The formed precipitate is collected by filtration, and is added to 30 mL of acetonitrile. Thereto 40 mL of DOWEX® 1X4, and 100 mL of water are added, and the mixture is stirred for 36 hours to dissolve the polymer. The solution is filtered by suction, and is concentrated to a volume of 50 mL. The concentrated matter is extruded through a (mol wt 10000)-cutoff filter (Millipore) at a nitrogen pressure of 275 kPa. Further, the extruded matter is passed with water as the solvent through a DOWEX® 1X4 column, and dialyzed in water. Thereby the polymer-(chloride salt) of Chemical Formula (1) is obtained.

(Preparation Example 17)

The process for synthesis of the complex polymer shown by Chemical Formula (2) is described below.

(Chemical Formula 2)



· · · (2)

To 6 mL of 1-vinylimidazole, is added 0.5 g of azobisisobutylonitrile. The mixture is allowed to react in an argon atmosphere at 70°C for 2 hours. The reaction solution is air-cooled. The formed precipitate is dissolved in methanol. The solution is added dropwise into acetone with violent agitation. The precipitate is collected by filtration to obtain poly-1-vinylimidazole. Separately, 0.76 g of 2,2′:6′2″-terpyridine and 1.42 g of ammonium hexachloroosmate are added to 5 mL of ethylene glycol, and the mixture is refluxed in an argon atmosphere for one hour. To this solution, 0.60 g of 4,4′-dimethyl-2,2′-bipyridine is added. The mixture is refluxed for 24 hours. The reaction solution is air-

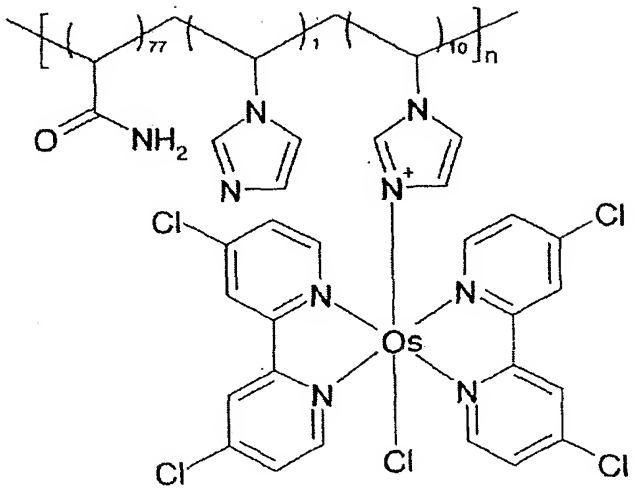
cooled. Impurity is removed by filtration. The filtrate is evaporated to remove the solvent to obtain osmium(2,2′:6′2″-terpyridine)(4,4′-dimethyl-2,2′-bipyridine) chloride salt.

A 200 mL portion of ethanol is added to 0.38 g of osmium(2,2′:6′2″-terpyridine)(4,4′-dimethyl-2,2′-bipyridine) chloride salt and 0.2 g of polyvinylimidazole. The mixture is refluxed in a nitrogen atmosphere for three days. The reaction mixture is filtered, and then the filtrate is added dropwise into 1 L of diethyl ether with violent agitation. The formed precipitate is recovered and dried to obtain the osmium complex represented by Chemical Formula (2). The compound is identified by elemental analysis.

(Preparation Example 18)

The process for synthesis of the complex polymer shown by Chemical Formula (3) is described below.

(Chemical Formula 3)



....(3)

To 7.5 mL of concentrated sulfuric acid, is added 1.9 g of 2,2'-bipyridyl-N,N'-dioxide. To the mixture, 1.6 g of fuming nitric acid is added gradually dropwise on a salted ice bath. The mixture is stirred for 5 minutes, and is poured onto crushed ice. The deposited solid is collected by filtration to obtain 4,4'-dinitro-2,2'-bipyridyl-N,N'-dioxide. A 0.5 g portion of this 4,4'-dinitro-2,2'-bipyridyl-N,N'-dioxide is added to 2.0 g of acetyl chloride, and the mixture is refluxed for one hour. The reaction solution is cooled, and an excess of acetyl chloride is distilled off. The reaction product is recrystallized from chloroform to obtain 4,4'-dichloro-2,2'-bipyridine. The product is identified by ^{1}H-NMR.

In 150 mL of water, are dissolved 24 g of acetylamide and 7 mL of 1-vinylimidazole. To the solution, is added an aqueous solution of 0.69 mL of N,N,N',N'-tetramethylethylenediamine in 50 mL of water, and is further added thereto an aqueous solution of 0.6 g of ammonium persulfate in 150 mL of water. The mixture is allowed to react in an argon atmosphere at 40°C for 30 minutes. Then the reaction solution is air-cooled, and the formed solid matter is allowed to precipitate in 2 L of methanol. The precipitate is dissolved again in 300 mL of water, and reprecipitated in 2 L of methanol. The precipitate is isolated, and is kept in methanol at 4°C for 12 hours. Thereafter, the solvent is evaporated under a reduced pressure to obtain a copolymer of polyacrylamide-polyvinylimidazole (7/1).

A 5 mL portion of ethylene glycol is added to 1.5 g of 4,4'-dichloro-2,2'-bipyridine and 1.4 g of ammonium hexachloroosmate, and the mixture is refluxed in an argon atmosphere for one hour. The reaction solution is air-cooled. Impurity is removed by filtration. The filtrate is evaporated to remove the solvent to obtain osmium(4,4'-dichloro-2,2'-bipyridine)2 dichloride.

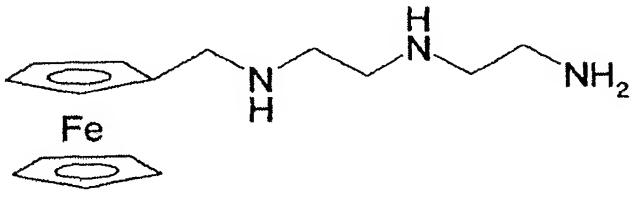
A 200 mL portion of ethanol is added to 1.0 g of osmium(4,4'-dichloro-2,2'-bipyridine)2 dichloride salt and 0.90 g of polyacrylamide-polyvinylimidazole

(7/1) copolymer. The mixture is refluxed in a nitrogen atmosphere for three days. The reaction mixture is filtered, and then the filtrate is added dropwise into 1 L of diethyl ether with violent agitation. The formed precipitate is recovered and dried to obtain the osmium complex represented by Chemical Formula (3). The compound is identified by elemental analysis.

(Preparation Example 19)

The process for synthesis of the ferrocene derivative shown by Chemical Formula (4), and the glucose oxidase modifying the ferrocene derivative is described below.

(Chemical Formula 4)



...(4)

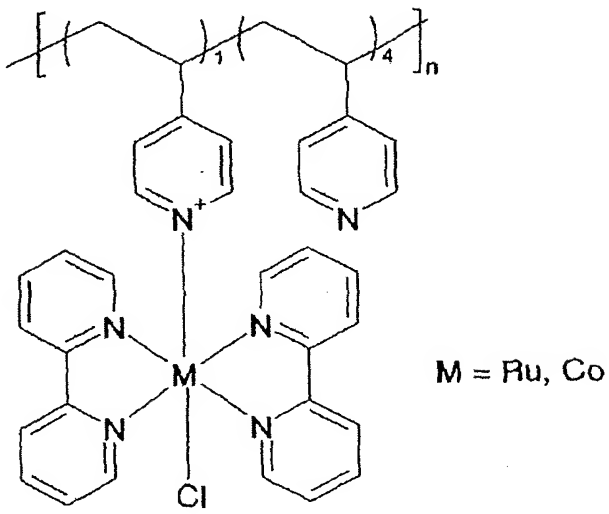
A 4.1 g portion of diethylenetriamine is dissolved in 200 mL of DMF. Thereto, is added a solution of 2.1 g ferrocene carbaldehyde in 100 mL of DMF. The mixture is stirred at 100°C for one hour. Thereto is added 1 g of sodium boron hydride saturated in water. The mixture is stirred at room temperature for one hour. The solvent is evaporated

off under a reduced pressure. The evaporation residue is treated by a silica column with a solvent of dichloromethane/methanol (10/1) to remove the dimer to obtain the ferrocene derivative compound represented by Chemical Formula (4). The compound is identified by ^{1}H-NMR. Separately, in a sample tube, 0.052 g of glucose oxidase (Aspergillus niger) is added to 1.3 mL of an aqueous 0.1M sodium hydrogencarbonate solution, and further thereto 0.7 mL of a 7mg/mL sodium periodate solution. The mixture is stirred in the dark for one hour. The solution is added to 2 mL of a 0.2M citrate buffer solution. Further thereto, 0.01 g of the ferrocene derivative compound represented by Chemical Formula (4) is added. The mixture is stirred for 15 hours, and centrifuged. The supernatant liquid is filtered through a 0.2μm-filter (Millipore), and is treated with a gel filtration column (Sephadex® G25) to eliminate unreacted ferrocene derivative to obtain a glucose oxidase combined with a ferrocene derivative.

(Preparation Example 20)

The complex polymer shown by Chemical Formula (5) below (M=Ru) is prepared by the process described below.

(Chemical Formula 5)



....(5)

A 20 mL portion of ethylene glycol is added to 0.21 g of ruthenium trichloride and 0.31 g of 2,2-bipyridine. The mixture is refluxed in an argon atmosphere for 24 hours. Thereafter the reaction solution is air-cooled. Impurity is eliminated by filtration, and the filtrate is evaporated by a reduced pressure to obtain ruthenium(2,2'-bipyridine)2 dichloride salt.

A 0.1 g portion of the ruthenium(2,2'-bipyridine)2 dichloride salt is added to a solution of 0.11 g of polyvinylpyridine (average mol wt: 150,000) in 30 mL of DMF. The mixture is stirred at 90°C for 24 hours, and thereafter is cooled to room temperature. The cooled mixture is poured gradually

into 1.2 L of ethyl acetate with violent agitation. Then the solvent is removed by decantation, and the solid matter is dissolved in methanol. The solution is filtered, and evaporated to a solution volume of about 200 mL. The formed product is reprecipitated in 1 L of diethyl ether. The product is vacuum-dried at 50°C for 24 hours, pulverized, and further dried for 48 hours to obtain the ruthenium complex polymer represented by Chemical Formula (5). The compound is identified by elemental analysis.

(Preparation Example 21)

The complex polymer shown by Chemical Formula (5) (M=Co) is prepared as below.

The cobalt complex shown by Chemical Formula (5) is prepared in the same manner as in Preparation Example 20 except that the ruthenium trichloride (0.21 g) is replaced by 0.13 g of cobalt dichloride, and the ruthenium(2,2'-bipyridine)2 dichloride salt (0.10 g) is replaced by 0.088 g of cobalt(2,2'-bipyridine)2 dichloride salt.

(Preparation Example 22)

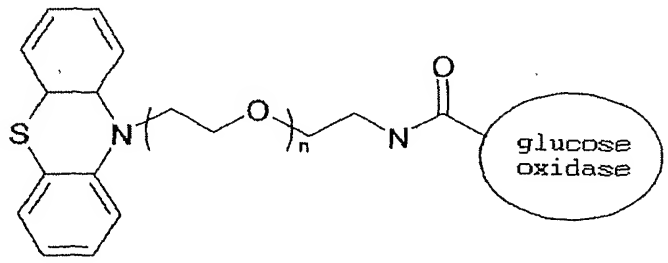
N^6-(2-aminoethyl)FAD is prepared through the process shown below. To an aqueous 10% FAD solution, is added an equimolar amount of ethylenimine. The pH is adjusted to 6-6.5. The mixture is allowed to react at 50°C for 6 hours. The reaction solution is cooled, and is added into ethanol on an ice bath to

cause precipitation. The precipitate is collected and is purified by anion exchange chromatography and reversed-phase high-speed chromatography to obtain purified N^6-(2-aminoethyl)FAD.

(Preparation Example 23)

The phenothiazine-modified glucose oxidase shown by Chemical Formula (6) blow is prepared through the process described below.

(Chemical Formula 6)



...(6)

To 50 mL of an aqueous 0.01M potassium hydroxide solution, are added 0.40 g of phenothiazine, and 3.0 g of polyethylene glycol (mol wt: 3000). Thereto 0.040 g of ethylene oxide is added with stirring on an ice bath. After stirring at ordinary temperature for 6 hours, the mixture is ultra-filtered to eliminate remaining unreacted phenotiazine. The filtrate is evaporated by vacuum to obtain polyethylene glycol-modified phenothiazine. A 3.2 g portion of this polyethylene glycol-modified phenothiazine is dissolved in 50 mL of

tetrahydrofuran (THF). Thereto 0.11 g of methanesulfonyl chloride, and 0.10 g of triethylamine are added. The mixture is stirred at room temperature for 2 hours. The solvent is evaporated to obtain methanesulfonylated polyethylene glycol-modified phenothiazine. This modified phenothiazine is dissolved in 100 mL of an aqueous 5% ammonia solution. The solution is stirred at room temperature for 2 days to obtain aminated polyethylene glycol-modified phenothiazine. Separately, glucose oxidase (Aspergillus niger) is treated with 10 mM of N-hydroxysuccinimide and 10 mM of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide in a phosphate buffer solution for activation of the carboxyl group on the surface thereof. Thereto the above aminated polyethylene glycol-modified phenothiazine is added and the mixture is stirred at 25°C for 24 hours. Therefrom the excess aminated polyethylene glycol-modified phenothiazine is eliminated by ultrafiltration to obtain the phenothiazine-modified glucose oxidase.

Enzyme preparation methods are described further.

(Preparation Example 24)

An FAD-free apoglucose oxidase is prepared through the process below. Glucose oxidase (Aspergillus niger) is dissolved in 3 mL of a 0.25M

sodium phosphate buffer solution (pH 6) containing 30% glycerol. This solution is cooled to 0°C, and the pH thereof is adjusted to 1.7 by addition of a 0.025M sodium phosphate buffer solution-sulfuric acid solution containing 30% glycerol (pH 1.1). This solution is allowed to pass through a Sephadex® G-25 column with a 0.1M sodium phosphate solution (pH: 1.7) containing 30% glycerol, and the intended fraction is recovered by monitoring with light of a wavelength of 280 nm. Dextran-coated charcoal is added to the recovered solution. The solution, after adjustment of pH to 7 by addition of a 1M sodium hydroxide solution, is stirred at 4°C for one hour. The resulting solution is centrifuged, passed through a 0.45μm filter, and dialyzed by use of a 0.1M sodium phosphate buffer solution to obtain the apoglucose oxidase.

(Preparation Example 25)

A cytochrome oxidase is prepared as shown below. One kilogram of minced and washed bovine heart muscle is agitated with 4 L of a 0.02M phosphate buffer solution (pH: 7.4) for 6 minutes. The mixture is centrifuged at 2500G for 20 minutes. The supernatant is recovered. The precipitate is stirred again with 2 L of a 0.02M phosphate buffer solution (pH: 7.4) for 3 minutes, and the stirred mixture is centrifuged at 2500G for 20 minutes. The supernatant is

recovered and is combined with the above-recovered supernatant. The pH of the combined supernatant is adjusted to 5.6. This liquid matter is centrifuged at 2500G for 20 minutes. The precipitate is dispersed again in 1 L of pure water, and centrifuged at 2500G for 20 minutes. The precipitate is dispersed again in 450 mL of a 0.02M phosphate buffer solution (pH: 7.4). Thereto 125 mL of a 10% NaCl solution, and 90 g of ammonium sulfate are added. The mixture is left standing at room temperature for two hours. A 41 g portion of ammonium sulfate is added thereto, and the mixture is centrifuged at 7000G for 20 minutes. To the recovered supernatant (500 mL), 50 g of ammonium sulfate is added, and the mixture is centrifuged at 7000G for 20 minutes. The precipitate is recovered, and is dissolved in 200 mL of a 0.1M phosphate buffer solution (pH: 7.4) containing 2% NaCl. A 66 mL portion of a saturated ammonium sulfate solution is added thereto. The mixture is left standing at 0°C for 12 hours. Thereafter the mixture is centrifuged at 7000G for 20 minutes. To the recovered supernatant (200 mL), 31 mL of an aqueous saturated ammonium sulfate solution is added. The mixture is centrifuged at 7000G for 20 minutes. The precipitate is recovered and is dissolved in 100 mL of a 0.1M phosphate buffer solution (pH: 7.4) containing 2% NaCl. The solution

is centrifuged at 7000G for 20 minutes to recover the precipitate. The precipitate is treated four times through steps: dissolution in 100 mL of a phosphate buffer solution; addition of 31 mL of an aqueous saturated ammonium sulfate solution; centrifuge; and precipitate recovery. Thereafter the recovered precipitate is dissolved in 30 mL of a 0.1M phosphate buffer solution (pH: 7.4) containing 1% Tween 80 to obtain a cytochrome oxidase solution.

(Preparation Example 26)

A commercial polystyrene type latex colloid dispersion liquid (Nippon Zeon Co.; average particle size: 100 nm) is employed. The dispersion medium of the dispersion liquid is replaced by ethanol. A cleaned gold substrate is allowed to stand in the dispersion liquid. The ethanol is allowed to evaporate at 30°C to obtain a porous film constituted of polystyrene spheres. This process is repeated several times to obtain a porous film constituted of polystyrene spheres of an intended film thickness (150 μm thick). The film is heated at 70°C for 30 minutes, and then washed with ethanol. Using this porous film as the working electrode and a platinum electrode as the counter electrode, electro-deposition is conducted in an aqueous 0.1M nickel sulfate solution at a current density of 0.1 mA/cm^2 by control with a galvanostat. The time of the

electro-deposition is controlled by monitoring the electrolysis current profile to obtain a film in a thickness nearly equivalent to the polystyrene film thickness. After the electro-deposition, the film is immersed in toluene for two days to remove the polystyrene spheres to obtain a conductive member constituted of nickel having numerous voids.

Methods for preparing a void size-gradient conductive member having numerous voids are described in Preparation Examples 27, 28, 29, 31, 33, 34, and 36. The diameters of particles can be measured by scanning electron microscopy, the sizes of the voids can be measured by gas adsorption measurement, and the film thicknesses can be measured by a surface roughness tester.

(Preparation Example 27)

Two grades of commercial polystyrene type latex colloid dispersion liquids (Nippon Zeon Co.; average particle sizes: 100 nm and 200 nm) are employed. The dispersion medium of the respective dispersion liquids is replaced by ethanol. Firstly, a cleaned gold substrate is allowed to stand in the dispersion liquid of the average particle size of 100 nm. The ethanol is allowed to evaporate at 30°C to obtain a porous film constituted of polystyrene spheres. This process is repeated several times to obtain a porous film constituted of 100-nm polystyrene spheres in an

intended film thickness (50 μm thick). Secondly, on the porous film of 100-nm polystyrene spheres, a porous film constituted of polystyrene spheres of the average particle size of 200 nm is formed in the same manner as the 100-nm polystyrene sphere film (about 100 μm thick, total thickness: about 150 μm). The film is heated at 70°C for 30 minutes, and then washed with ethanol. Thereafter, by using this porous film, a void size-gradient conductive member having numerous voids is prepared in the same manner as in Preparation Example 26.

(Preparation Example 28)

Three grades of commercial polystyrene type latex colloid dispersion liquids (Nippon Zeon Co.; average particle sizes: 100 nm, 200 nm, and 300 nm) are employed. The dispersion medium of the respective dispersion liquids is replaced by ethanol. Firstly, a cleaned gold substrate is allowed to stand in the dispersion liquid of the average particle size of 100 nm. The ethanol is allowed to evaporate at 30°C to obtain a porous film constituted of polystyrene spheres. This process is repeated several times to obtain a porous film constituted of 100-nm polystyrene spheres in an intended film thickness (about 50 μm thick). Secondly, on the porous film of 100-nm styrene spheres, a porous film constituted of polystyrene spheres of the average

particle size of 200 nm is formed in the same manner as the 100-nm styrene sphere film (about 50 μm thick, total film thickness: about 100 μm). Thirdly, on the porous films of 100-nm and 200-nm styrene spheres, a porous film constituted of polystyrene spheres of the average particle size of 300 nm is formed in the same manner as the 100-nm styrene sphere film (about 50 μm thick, total thickness: about 150 μm). The film is heated at 70°C for 30 minutes, and then washed with ethanol. Thereafter, by using this porous film, a void size-gradient conductive member having numerous voids is prepared in the same manner as in Preparation Example 26.

(Preparation Example 29)

Two grades of commercial silica colloid dispersion liquids (Nissan Chemical Ind.; average particle sizes: 100 nm, and 300 nm) are employed. The dispersion medium of the respective dispersion liquids is replaced by ethanol. Firstly, a cleaned gold substrate is allowed to stand in the dispersion liquid of the average particle size of 100 nm. The ethanol is allowed to evaporate at 30°C to obtain a porous film constituted of silica spheres. This process is repeated several times to increase the thickness of a porous film constituted of silica spheres (about 50 nm thick). Secondly, on the porous film of 100-nm silica sphere film formed above, a

porous film constituted of silica spheres of average particle size of 300 nm is formed in the same manner as the formation of the 100-nm porous film (about 50 μm thick, total thickness: about 100 μm). The film is heated at 200°C for three hours, and then washed with ethanol. In a three-electrode cell, with this porous film as the working electrode, a platinum electrode as the counter electrode, and an Ag/AgCl electrode as the reference electrode, electrolytic polymerization is conducted in a solution of 0.1M 3,4,-ethylenedioxythiophene and 0.1M lithium perchlorate in acetonitrile at a potential of 1.1 V (vs Ag/AgCl) by control with a potentiostat. The time of the polymerization is controlled by monitoring the electrolysis current profile to obtain a film in a thickness nearly equivalent to the silica sphere porous film thickness. After the electrolytic polymerization, the film is immersed in a 20% hydrofluoric acid solution for two days to remove the silica spheres to obtain a void size-gradient conductive member (100 μm thick) constituted of poly(3,4-ethylenedoxythiophene), an electroconductive polymer, having numerous voids.

(Preparation Example 30)

Commercial fine particulate electroconductive titanium oxide (Titan Kogyo K.K.; particle diameter: about 250 nm) is dispersed in terpinol. The

viscosity of the dispersion is adjusted by addition of ethylcellulose to obtain a titanium oxide paste. This titanium oxide paste is applied on a cleaned gold substrate by screen process printing, and is sintered at 450°C for one hour to obtain a sintered porous titanium oxide film (100 μm thick). Using this porous film as the working electrode and a platinum wire as the counter electrode, electrolytic plating is conducted in a gold plating solution (Kamimura Kogyo K.K.; 535LC) in a jet flow of said gold-plating solution with an ultrasonic vibration at a current density of 0.1 mA/cm^2 by control with a galvanostat for one hour to obtain a conductive member constituted of gold-plated porous titanium oxide having numerous voids.

(Preparation Example 31)

Two grades of commercial fine particulate electroconductive titanium oxide (Titan Kogyo K.K.; particle sizes: about 250 nm, and 400 nm) are respectively dispersed in terpinol, and the viscosities are respectively adjusted by addition of ethylcellulose to obtain titanium oxide pastes. The titanium oxide paste of the particle size of 250 nm is firstly applied on a cleaned gold substrate by screen process printing (in a sintered thickness of about 50 μm) and calcined at 150°C for 5 minutes. Thereon, the titanium oxide paste of the particle size of about 400 nm is applied, and the paste is

sintered at 450°C for one hour to obtain a sintered porous titanium oxide film (total thickness: 100 μm). By use of this porous film, a void size-gradient conductive member constituted of gold-plated porous titanium oxide having numerous voids is prepared in the same manner as in Preparation Example 29.

(Preparation Example 32)

Three sheets of a foamed nickel alloy (Mitsubishi Materials Corp.; MA600; thickness: 0.5 mm, pore size: 50 μm) are superposed and bonded by spot welding to obtain a conductive member having numerous voids constituted of the nickel alloy.

(Preparation Example 33)

Two types of foamed nickel alloy sheets (Mitsubishi Materials Corp.; MA600; thickness: 0.5 mm, pore sizes: 50 μm, and 150 μm) are employed. One sheet of the pore size of 50 μm, and two sheets of the pore size of 150 μm are superposed in this order (three sheets in total), and bonded by spot welding to obtain a void size-gradient conductive member constituted of the nickel alloy having numerous voids.

(Preparation Example 34)

Three types of foamed nickel alloy sheets (Mitsubishi Materials Corp.; MA600; thickness: 0.5 mm, pore sizes: 50 μm, 150 μm, and 300 μm) are employed. The sheet of the pore size of 50 μm, the sheet of the pore size of 150 μm, and the sheet of the pore size

of 300 μm are superposed in this order (three sheets in total), and bonded by spot welding to obtain a void size-gradient conductive member constituted of the nickel alloy having numerous voids.

(Preparation Example 35)

Two sheets of carbon fiber (Toray Ind.; Toreca Cloth; thickness: 0.2 mm; fiber density: 40 fibers/25cm) are superposed, and are cut in 1 cm square. The cut sheets are united by applying carbon paste (SPI Co.) on four side peripheries to obtain a conductive member constituted of carbon fiber and having numerous voids.

(Preparation Example 36)

Two types of sheets of carbon fiber (Toray Ind.; Toreca Cloth; thickness: 0.2 mm; fiber density: 40 fibers/25cm and 22.5 fibers/25cm) are superposed, and are cut in 1 cm square. The cut sheets are united by applying carbon paste (SPI Co.) on four side peripheries to obtain a void size-gradient conductive member constituted of carbon fiber having numerous voids.

The process for producing the enzyme electrode of the present invention is described below.

(Example 1)

A sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp.; SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and

Au); thickness: 0.5 mm; gold plating thickness 0.5 μm; pore size: 50 μm) is cut in 1 cm square; washed and dried; and subjected to UV-ozone treatment for hydrophilicity. An electrolytic solution is prepared by mixing 1 mL of an aqueous solution containing 1.0 mg/mL of glucose oxidase (Aspergillus niger) and 1 wt% Triton X-100® and 9 mL of an aqueous solution of 0.1M pyrrole and 0.1M lithium perchlorate. Electrolytic polymerization is conducted with the electrolytic solution with the above foamed metal as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl electrode as the reference electrode in a nitrogen atmosphere by applying 100 pulses of 1.1 V (vs Ag/AgCl) for one second and 0.35 V for 30 seconds. The working electrode after the electrolytic polymerization is washed with water to obtain a glucose-oxidase enzyme electrode containing polypyrrole serving simultaneously as the carrier and the mediator (carrier-and-mediator).

(Example 2)

An alcohol-dehydrogenase enzyme electrode employing polypyrrole as the carrier-and-mediator is prepared in the same manner as in Example 1 except that 245 U/mL of quinohemoprotein-alcohol dehydrogenase (Gluconobacter sp-33) is used instead of 1.0 mg/mL of glucose oxidase (Aspergillus niger).

(Example 3)

A glucose-oxidase enzyme electrode employing poly(3,4-ethylenedioxythiophene) as the carrier-and-mediator is prepared in the same manner as in Example 1 except that 3,4-ethylenedioxythiophene is used instead of pyrrole.

(Example 4)

An alcohol-dehydrogenase enzyme electrode employing poly(3,4-ethylenedioxythiophene) as the carrier-and-mediator is prepared in the same manner as in Example 2 except that 3,4-ethylenedioxythiophene is used instead of pyrrole.

(Example 5)

A glucose-oxidase enzyme electrode employing polyaniline as the carrier-and-mediator is prepared in the same manner as in Example 1 except that aniline is used instead of pyrrole.

(Example 6)

An alcohol-dehydrogenase enzyme electrode employing polyaniline as the carrier-and-mediator is prepared in the same manner as in Example 2 except that aniline is used instead of pyrrole.

(Example 7)

In 5 mL of water in a sample tube, the osmium polymer prepared in Preparation Example 17 is dissolved at a concentration of 10 mg/mL. Thereto, 1 mL of a 0.2M citrate buffer solution, and 1 mL of an aqueous solution of 30mg/mL laccase (Coriolus

hirsutus) are added, and the mixture is stirred. Thereto, 2 mL of an aqueous 10 mg/mL polyethylene glycol diglycidyl ether solution is added and the mixture is stirred. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared enzyme-osmium polymer solution, taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 8)

In 5 mL of water in a sample tube, the osmium polymer prepared in Preparation Example 18 is dissolved at a concentration of 10 mg/mL. Thereto, are added 1 mL of a phosphate buffer solution, 1 mL of an aqueous 46mg/mL bilirubin oxidase solution, and 1 mL of an aqueous 7 mg/mL polyethylene glycol diglycidyl ether solution. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in

the above prepared enzyme-osmium polymer solution, taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 9)

In a sample tube, is prepared 1 mL of an aqueous 40mg/mL solution of ferrocene-modified glucose oxidase shown in Preparation Example 19 in 0.1M sodium hydrogencarbonate. Thereto, 0.5 mL of an aqueous 7mg/mL sodium periodate solution is added, and the mixture is stirred in the dark for one hour. Thereto, are added 6 mL of an aqueous 4mg/mL polyvinylimidazole solution and 0.4 mL of an aqueous 2.5mg/mL polyethylene glycol diglycidyl ether solution. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared modified enzyme solution, taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 10)

In a sample tube, is prepared 1 mL of an aqueous solution containing 40 mg/mL glucose oxidase (Aspergillus niger) and 0.1M sodium hydrogen carbonate. Thereto, 0.5 mL of an aqueous 7mg/mL

sodium periodate solution is added, and the mixture is stirred in the dark for one hour. Thereto, are added 6 mL of an aqueous 10mg/mL solution of the ruthenium complex polymer prepared in Preparation Example 20 and 0.4 mL of an aqueous 2.5mg/mL polyethylene glycol diglycidyl ether solution. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared modified enzyme solution, taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 11)

An enzyme electrode is prepared in the same manner as in Example 10 except that the cobalt complex polymer shown in Preparation Example 21 is used instead of the ruthenium complex polymer of Preparation Example 20.

(Example 12)

Into 5 mL of a phosphate buffer solution, are added 34 units of glucose dehydrogenase (Thermoplasma acidophilum), 27 units of diaphorase (Spinacia oleracea), 0.22 mg of vitamin K3, 0.15 mg of nicotinamide adenine dinucleotide (NADH), and 0.13 mg

of polyvinylpyridine (average mol wt: 150,000). Thereto 0.4 mL of an aqueous 2.5mg/mL polyethylene glycol diglycidyl ether solution, and the mixture is stirred. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared modified enzyme solution, taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 13)

An enzyme electrode is prepared in the same manner as in Example 12 except that 0.27 mg of anthraquinone is used instead of 0.22 mg of vitamin K3.

(Example 14)

An enzyme electrode is prepared in the same manner as in Example 9 except that the phenothiazine-modified glucose oxidase shown in Preparation Example 23 is used instead of the ferrocene-modified glucose oxidase of Preparation Example 19.

(Example 15)

In a sample tube, is prepared 1 mL of an aqueous solution containing 40 mg/mL glucose oxidase (Aspergillus niger) and 0.1M sodium hydrogencarbonate.

Thereto, 0.5 mL of an aqueous 7mg/mL sodium periodate solution is added, and the mixture is stirred in the dark for one hour. Thereto, are added 6 mL of an aqueous 10mg/mL solution of the osmium complex polymer prepared in Preparation Example 16 and 0.4 mL of an aqueous 2.5mg/mL polyethylene glycol diglycidyl ether solution. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared modified enzyme solution, then taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 16)

To 1.8 mL of a 0.1M phosphate buffer solution, are added 0.25 mL of a 1M N-(3-(trimethoxysilyl)propyl)ethylenediamine solution and 0.25 mL of a 0.01M chlorauric acid solution. The mixture is irradiated with an ultrasonic wave for 10 minutes. Hydrochloric acid is added to the mixture to adjust the pH to 7, and 0.013 mL of a 0.1M sodium boron hydride solution is added thereto. The resulting sol is stirred for 24 hours to prepare a silica sol containing fine particulate gold.

Separately, 10 mg of glucose oxidase is dissolved in 6 mL of a 0.05M phosphate buffer solution (pH: 7.0). Therein 1.6 g of polyvinylpyridine is added and mixed uniformly. The resulting mixture solution is added to the above obtained silica sol containing fine particulate gold uniformly by stirring. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared mixture solution, then taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 17)

An enzyme electrode is prepared in the same manner as in Example 16 except that palladium chloride is used instead of the chloroauric acid.

(Example 18)

In a nitrogen atmosphere, 0.25 mL of titanium(IV) isopropoxide is dissolved in a small amount of isopropanol. Thereto, 1.8 mL of a 0.1M phosphate buffer solution and 0.25 mL of a 0.01M chloroauric acid solution are added. The resulting mixture is irradiated with ultrasonic wave for one hour. The pH of the mixture is adjusted to 7 by

addition of 0.1M hydrochloric acid. Thereto 0.013 mL of a 0.1M sodium boron hydride is added, and the mixture is stirred for 24 hours to obtain titania sol containing fine particulate gold. Separately, 10 mg of glucose oxidase is dissolved in 6 mL of a 0.05M phosphate buffer solution (pH: 7.0) and 1.6 g of polyvinylpyridine is added thereto and stirred uniformly. This mixture is added to the above prepared titania sol containing fine particulate gold. The resulting mixture is stirred uniformly. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared mixture solution, then taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 19)

An enzyme electrode is prepared in the same manner as in Example 18 except that palladium chloride is used instead of the chloroauric acid.

(Example 20)

In 8 mL of a 0.1M phosphate buffer solution, is dissolved 20 mg of polylysine hydrochloride (average mol wt: 70,000). Thereto are added 40 mg of

bilirubin oxidase and 27 mg of potassium octacyanotungstate. The mixture is stirred at 0°C for one hour. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in the above prepared solution, then taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 21)

Into 5 mL of a phosphate buffer solution, are added 34 units of glucose dehydrogenase (Thermoplasma acidophilum), 27 units of diaphorase (Spinacia oleracea), 0.22 mg of vitamin K3, and 0.15 mg of NADH; and further 0.5 mL of 1% bovin serum albumin, and 0.4 mL of a 2.5mg/mL glutalaldehyde solution. The mixture is stirred. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in an aqueous 0.02M aminoethanethiol solution for 2 hours, then taken out and washed with water. Thereafter the

aminoethanethiol-treated sheet is immersed in the above prepared enzyme solution, then taken out, and dried in a desiccator for two days to obtain an enzyme electrode.

(Example 22)

A sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in an aqueous 0.02M cystamine solution for 2 hours, then taken out, and washed with water to prepare a cystamine-modified electrode. This cystamine-modified electrode is immersed in a 0.01M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer solution containing 3mM pyrroloquinolinequinone (PQQ) and 10 mM of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide for one hour and washed with water to modify the electrode with PQQ. Further, this PQQ-modified electrode is immersed in a 0.01M HEPES buffer solution (pH: 7.3) containing 1 mM N^6-(2-aminoethyl)FAD described in Preparation Example 22 and 10 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide for 2 hours and washed with water to modify the electrode with FAD. Further, this modified electrode is immersed in a

0.1M phosphate buffer solution (pH: 7.0) containing 4 mg/mL of the apoglucose oxidase described in Preparation Example 24 at 25°C for 4 hours, and at 4°C for 12 hours, then taken out, and further immersed in a phosphate buffer solution (pH: 7.0) to prepare an enzyme electrode.

(Example 23)

A 0.06 mM portion of fine particulate gold (Nanoprobes) modified by sulfo-N-hydroxysuccinimide, and 0.68mM of N^6-(2-aminoethyl)FAD described in Preparation Example 22 dissolved in 0.01M HEPES buffer solution (pH: 7.9) are stirred at room temperature for one hour and 4°C for 12 hours to allow the fine particulate gold and the N^6-(2-aminoethyl)FAD to react. The unreacted N^6-(2-aminoethyl)FAD is eliminated by Spin Column (Sigma) to prepare fine particulate FAD-modified gold. Further, 3 mg/mL of apoglucose oxidase described in Preparation Example 24, and 4.8 μM of the above FAD-modified fine particulate gold are stirred in a 0.1M phosphate buffer solution containing 30% glycerol, 0.1% bovin serum albumin, and 0.1% sodium azide at room temperature for 4 hours and at 4°C for 12 hours. Then resulting glucose oxidase-modified fine particulate gold is separated by centrifugation. Separately, a sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L

(constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in an aqueous 0.02M cystamine solution for 2 hours, then taken out, and washed with water to prepare a cystamine-modified electrode. Thereafter the cystamine-modified electrode is immersed in a 1μM solution of glucose oxidase-modified fine particulate gold in a phosphate buffer solution at 4°C for 12 hours to prepare an enzyme electrode.

(Example 24)

A sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in a 1mM cystamine solution in ethanol for 2 hours, taken out, and washed with water to prepare a cystamine-modified base plate. This base plate is immersed in a solution of 1mM 1,2-dehydro-1,2-methanofullerene[60]-61-carboxylic acid (Material Technologies Research Limited) and 5mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in ethanol:dimethylsulfoxide (DMSO) (1:1) at room

temperature for 4 hours, and washed with ethanol:DMSO mixed solvent to prepare a fullerene-modified base plate. Separately 0.8 mL of a 2.5mg/mL glutaraldehyde solution is added to 10 mL of a 30 mg/mL glucose oxidase (Aspergillus niger) in a phosphate buffer solution and stirred. In this solution, the above fullerene-modified base plate is immersed at room temperature for one hour and at 4°C for 12 hours, then taken out, and washed with a phosphate buffer solution, and dried in a desiccator for 2 days to prepare an enzyme electrode.

(Example 25)

A sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in an aqueous 0.02M cystamine solution for 2 hours, then taken out, and washed with water. This sheet is immersed in a solution of 0.3mM microperoxidase 11 (MP11) and 10mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in 0.01M HEPES buffer solution for three hours, then taken out, and immersed in a 0.01M HEPES buffer solution (pH: 7.3) for one hour to prepare an enzyme electrode.

(Example 26)

A sheet of gold-plated foamed stainless steel (Mitsubishi Materials Corp., SUS316L (constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, S, and Au), thickness: 0.5 mm, gold-plating thickness: 0.5 μm, pore size: 50 μm) is cut in 1 cm square, cleaned, and subjected to UV-ozone treatment. This cut sheet is immersed in an aqueous 0.02M cystamine solution for 2 hours, then taken out, and washed with water to prepare a cystamine-modified electrode. This cystamine-modified electrode is immersed in a 0.01M HEPES buffer solution containing 3mM N-succinimidyl-3-maleimidopropionate and 10mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide for one hour, and is washed with a 0.01M HEPES buffer solution for modification. This electrode is immersed in a 0.1M phosphate buffer solution (pH: 7.0) containing 4mg/mL cytochrome C at 25°C for 4 hours and at 4°C for 12 hours, then taken out, and immersed in a phosphate buffer solution (pH: 7.0) for one hour to modify the maleimide by the thiol group of the enzyme. Further, this electrode is immersed in a 0.1M phosphate buffer solution (pH: 7.0) containing 4mg/mL cytochorme oxidase described in Preparation Example 25 at 25°C for 4 hours and at 4°C for 12 hours, then taken out, and immersed in a phosphate buffer solution (pH: 7.0) for one hour to couple the cytochrome C with the cytochrome oxidase. Then the electrode is immersed

in a 10mM glutaraldehyde solution in 0.1M phosphate buffer solution (pH: 7.0) at 25°C for 10 minutes and 4°C for one hour to obtain an immobilized-enzyme electrode.

(Example 27)

An enzyme electrode is prepared in the same manner as in Example 4 except that a foamed nickel alloy (Mitsubishi Materials Corp., constituting elements: Ni, Cr, Ti, Nb, Al, Mn, Si, and C; thickness: 0.5 mm; gold-plating thickness: 0.5 μm, pore size: 50 μm) is used instead of the gold-plated foamed stainless steel.

(Example 28)

An enzyme electrode is prepared in the same manner as in Example 8 except that a foamed nickel alloy (Mitsubishi Materials Corp., constituting elements: Ni, Cr, Ti, Nb, Al, Mn, Si, and C; thickness: 0.5 mm; gold-plating thickness: 0.5 μm, pore size: 50 μm) is used instead of the gold-plated foamed stainless steel.

(Example 29)

An enzyme electrode is prepared in the same manner as in Example 15 except that a foamed nickel alloy (Mitsubishi Materials Corp., constituting elements: Ni, Cr, Ti, Nb, Al, Mn, Si, and C; thickness: 0.5 mm; gold-plating thickness: 0.5 μm, pore size: 50 μm) is used instead of the gold-plated

foamed stainless steel.

(Example 30)

An enzyme electrode is prepared in the same manner as in Example 18 except that a foamed nickel alloy (Mitsubishi Materials Corp., constituting elements: Ni, Cr, Ti, Nb, Al, Mn, Si, and C; thickness: 0.5 mm; gold-plating thickness: 0.5 μm; pore size: 50 μm) is used instead of the gold-plated foamed stainless steel.

(Example 31)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing nickel described in Preparation Example 1 is used instead of the gold-plated foamed stainless steel.

(Example 32)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing nickel described in Preparation Example 1 is used instead of the gold-plated foamed stainless steel.

(Example 33)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing nickel described in Preparation Example 1 is used instead of the gold-plated foamed stainless steel.

(Example 34)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing nickel described in Preparation Example 1 is used instead of the gold-plated foamed stainless steel.

(Example 35)

An enzyme electrode is prepared in the same manner as in Example 4 except that a stainless steel net (Nilaco; constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, and S; 400 mesh) is used instead of the gold-plated foamed stainless steel.

(Example 36)

An enzyme electrode is prepared in the same manner as in Example 8 except that a stainless steel net (Nilaco; constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, and S; 400 mesh) is used instead of the gold-plated foamed stainless steel.

(Example 37)

An enzyme electrode is prepared in the same manner as in Example 15 except that a stainless steel net (Nilaco; constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, and S; 400 mesh) is used instead of the gold-plated foamed stainless steel.

(Example 38)

An enzyme electrode is prepared in the same manner as in Example 18 except that a nickel alloy

net (Nilaco; constituting elements: Fe, Cr, Ni, Mo, Si, O, Mn, C, P, and S; 400 mesh) is used instead of the gold-plated foamed stainless steel.

(Example 39)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing platinum described in Preparation Example 2 is used instead of the gold-plated foamed stainless steel.

(Example 40)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing platinum described in Preparation Example 2 is used instead of the gold-plated foamed stainless steel.

(Example 41)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing platinum described in Preparation Example 2 is used instead of the gold-plated foamed stainless steel.

(Example 42)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing platinum described in Preparation Example 2 is used instead of the gold-plated foamed stainless steel.

(Example 43)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing gold described in Preparation Example 3 is used instead of the gold-plated foamed stainless steel.

(Example 44)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing gold described in Preparation Example 3 is used instead of the gold-plated foamed stainless steel.

(Example 45)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing gold described in Preparation Example 3 is used instead of the gold-plated foamed stainless steel.

(Example 46)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing gold described in Preparation Example 3 is used instead of the gold-plated foamed stainless steel.

(Example 47)

An enzyme electrode is prepared in the same manner as in Example 24 except that the conductive

member constituted of void-containing gold described in Preparation Example 3 is used instead of the gold-plated foamed stainless steel.

(Example 48)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing palladium described in Preparation Example 4 is used instead of the gold-plated foamed stainless steel.

(Example 49)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing palladium described in Preparation Example 4 is used instead of the gold-plated foamed stainless steel.

(Example 50)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing palladium described in Preparation Example 4 is used instead of the gold-plated foamed stainless steel.

(Example 51)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing palladium described in Preparation Example 4 is used instead of the gold-plated foamed stainless steel.

(Example 52)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of a void-containing polypyrrole electrode described in Preparation Example 5 is used instead of the gold-plated foamed stainless steel.

(Example 53)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of a void-containing polypyrrole electrode described in Preparation Example 5 is used instead of the gold-plated foamed stainless steel.

(Example 54)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of a void-containing polypyrrole electrode described in Preparation Example 5 is used instead of the gold-plated foamed stainless steel.

(Example 55)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of a void-containing polypyrrole electrode described in Preparation Example 5 is used instead of the gold-plated foamed stainless steel.

(Example 56)

An enzyme electrode is prepared in the same manner as in Example 1 except that the conductive

member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 57)

An enzyme electrode is prepared in the same manner as in Example 2 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 58)

An enzyme electrode is prepared in the same manner as in Example 3 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 59)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 60)

An enzyme electrode is prepared in the same

manner as in Example 5 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 61)

An enzyme electrode is prepared in the same manner as in Example 6 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 62)

An enzyme electrode is prepared in the same manner as in Example 7 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 63)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 64)

An enzyme electrode is prepared in the same manner as in Example 9 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 65)

An enzyme electrode is prepared in the same manner as in Example 10 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 66)

An enzyme electrode is prepared in the same manner as in Example 11 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 67)

An enzyme electrode is prepared in the same manner as in Example 12 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 68)

An enzyme electrode is prepared in the same manner as in Example 13 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 69)

An enzyme electrode is prepared in the same manner as in Example 14 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 70)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 71)

An enzyme electrode is prepared in the same manner as in Example 16 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed

stainless steel.

(Example 72)

An enzyme electrode is prepared in the same manner as in Example 17 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 73)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 74)

An enzyme electrode is prepared in the same manner as in Example 19 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 75)

An enzyme electrode is prepared in the same manner as in Example 20 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation

Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 76)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) described in Preparation Example 7 is used instead of the gold-plated foamed stainless steel.

(Example 77)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) described in Preparation Example 7 is used instead of the gold-plated foamed stainless steel.

(Example 78)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) described in Preparation Example 7 is used instead of the gold-plated foamed stainless steel.

(Example 79)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing poly(3,4-

ethylenedioxythiophene)-poly(styrenesulfonate) described in Preparation Example 7 is used instead of the gold-plated foamed stainless steel.

(Example 80)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing polyaniline described in Preparation Example 8 is used instead of the gold-plated foamed stainless steel.

(Example 81)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing polyaniline described in Preparation Example 8 is used instead of the gold-plated foamed stainless steel.

(Example 82)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing polyaniline described in Preparation Example 8 is used instead of the gold-plated foamed stainless steel.

(Example 83)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing polyaniline described in Preparation Example 8 is used instead of the gold-plated foamed stainless steel.

(Example 84)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing ITO described in Preparation Example 9 is used instead of the gold-plated foamed stainless steel.

(Example 85)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing ITO described in Preparation Example 9 is used instead of the gold-plated foamed stainless steel.

(Example 86)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing ITO described in Preparation Example 9 is used instead of the gold-plated foamed stainless steel.

(Example 87)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing ITO described in Preparation Example 9 is used instead of the gold-plated foamed stainless steel.

(Example 88)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void-

containing conductive member constituted of gold-plated porous titanium oxide described in Preparation Example 10 is used instead of the gold-plated foamed stainless steel.

(Example 89)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void-containing conductive member constituted of gold-plated porous titanium oxide described in Preparation Example 10 is used instead of the gold-plated foamed stainless steel.

(Example 90)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void-containing conductive member constituted of gold-plated porous titanium oxide described in Preparation Example 10 is used instead of the gold-plated foamed stainless steel.

(Example 91)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void-containing conductive member constituted of gold-plated porous titanium oxide described in Preparation Example 10 is used instead of the gold-plated foamed stainless steel.

(Example 92)

An enzyme electrode is prepared in the same

manner as in Example 24 except that the void-containing conductive member constituted of gold-plated porous titanium oxide described in Preparation Example 10 is used instead of the gold-plated foamed stainless steel.

(Example 93)

An enzyme electrode is prepared in the same manner as in Example 1 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 94)

An enzyme electrode is prepared in the same manner as in Example 2 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 95)

An enzyme electrode is prepared in the same manner as in Example 3 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 96)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 97)

An enzyme electrode is prepared in the same manner as in Example 5 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 98)

An enzyme electrode is prepared in the same manner as in Example 6 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 99)

An enzyme electrode is prepared in the same manner as in Example 7 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 100)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 101)

An enzyme electrode is prepared in the same manner as in Example 9 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 102)

An enzyme electrode is prepared in the same manner as in Example 10 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 103)

An enzyme electrode is prepared in the same manner as in Example 11 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-

plated foamed stainless steel.

(Example 104)

An enzyme electrode is prepared in the same manner as in Example 12 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 105)

An enzyme electrode is prepared in the same manner as in Example 13 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 106)

An enzyme electrode is prepared in the same manner as in Example 14 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 107)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in

Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 108)

An enzyme electrode is prepared in the same manner as in Example 16 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 109)

An enzyme electrode is prepared in the same manner as in Example 17 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 110)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 111)

An enzyme electrode is prepared in the same manner as in Example 19 except that the void-containing conductive member constituted of carbon-

coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 112)

An enzyme electrode is prepared in the same manner as in Example 20 except that the void-containing conductive member constituted of carbon-coated needle-crystalline zinc oxide described in Preparation Example 11 is used instead of the gold-plated foamed stainless steel.

(Example 113)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void-containing conductive member constituted of alumina having nanoholes described in Preparation Example 12 is used instead of the gold-plated foamed stainless steel.

(Example 114)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void-containing conductive member constituted of alumina having nanoholes described in Preparation Example 12 is used instead of the gold-plated foamed stainless steel.

(Example 115)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void-

containing conductive member constituted of alumina having nanoholes described in Preparation Example 12 is used instead of the gold-plated foamed stainless steel.

(Example 116)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void-containing conductive member constituted of alumina having nanoholes described in Preparation Example 12 is used instead of the gold-plated foamed stainless steel.

(Example 117)

An enzyme electrode is prepared in the same manner as in Example 24 except that the void-containing conductive member constituted of alumina having nanoholes described in Preparation Example 12 is used instead of the gold-plated foamed stainless steel.

(Example 118)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void-containing conductive member constituted of graphite particles having numerous voids described in Preparation Example 13 is used instead of the gold-plated foamed stainless steel.

(Example 119)

An enzyme electrode is prepared in the same

manner as in Example 8 except that the void-containing conductive member constituted of graphite particles having numerous voids described in Preparation Example 13 is used instead of the gold-plated foamed stainless steel.

(Example 120)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void-containing conductive member constituted of graphite particles having numerous voids described in Preparation Example 13 is used instead of the gold-plated foamed stainless steel.

(Example 121)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void-containing conductive member constituted of graphite particles having numerous voids described in Preparation Example 13 is used instead of the gold-plated foamed stainless steel.

(Example 122)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void-containing conductive member constituted of carbon black particles having numerous voids described in Preparation Example 14 is used instead of the gold-plated foamed stainless steel.

(Example 123)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void-containing conductive member constituted of carbon black particles having numerous voids described in Preparation Example 14 is used instead of the gold-plated foamed stainless steel.

(Example 124)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void-containing conductive member constituted of carbon black particles having numerous voids described in Preparation Example 14 is used instead of the gold-plated foamed stainless steel.

(Example 125)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void-containing conductive member constituted of carbon black particles having numerous voids described in Preparation Example 14 is used instead of the gold-plated foamed stainless steel.

(Example 126)

An enzyme electrode is prepared in the same manner as in Example 1 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 127)

An enzyme electrode is prepared in the same manner as in Example 2 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 128)

An enzyme electrode is prepared in the same manner as in Example 3 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 129)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 130)

An enzyme electrode is prepared in the same manner as in Example 5 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 131)

An enzyme electrode is prepared in the same manner as in Example 6 except that the void-containing conductive member constituted of carbon

nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 132)

An enzyme electrode is prepared in the same manner as in Example 7 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 133)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 134)

An enzyme electrode is prepared in the same manner as in Example 9 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 135)

An enzyme electrode is prepared in the same manner as in Example 10 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 136)

An enzyme electrode is prepared in the same manner as in Example 11 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 137)

An enzyme electrode is prepared in the same manner as in Example 12 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 138)

An enzyme electrode is prepared in the same manner as in Example 13 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 139)

An enzyme electrode is prepared in the same manner as in Example 14 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 140)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void-containing conductive member constituted of carbon

nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 141)

An enzyme electrode is prepared in the same manner as in Example 16 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 142)

An enzyme electrode is prepared in the same manner as in Example 17 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 143)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 144)

An enzyme electrode is prepared in the same manner as in Example 19 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 145)

An enzyme electrode is prepared in the same manner as in Example 20 except that the void-containing conductive member constituted of carbon nanotubes described in Preparation Example 15 is used instead of the gold-plated foamed stainless steel.

(Example 146)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void-containing conductive member constituted of nickel having numerous voids described in Preparation Example 26 is used instead of the gold-plated foamed stainless steel.

(Example 147)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void-containing conductive member constituted of nickel having numerous voids described in Preparation Example 26 is used instead of the gold-plated foamed stainless steel.

(Example 148)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void-containing conductive member constituted of nickel having numerous voids described in Preparation Example 26 is used instead of the gold-plated foamed stainless steel.

(Example 149)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void-containing conductive member constituted of nickel having numerous voids described in Preparation Example 26 is used instead of the gold-plated foamed stainless steel.

(Example 150)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 27 is used instead of the gold-plated foamed stainless steel.

(Example 151)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 27 is used instead of the gold-plated foamed stainless steel.

(Example 152)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 27 is used instead of the gold-plated foamed stainless steel.

(Example 153)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 27 is used instead of the gold-plated foamed stainless steel.

(Example 154)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 28 is used instead of the gold-plated foamed stainless steel.

(Example 155)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 28 is used instead of the gold-plated foamed stainless steel.

(Example 156)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 28 is used instead of the gold-plated foamed

stainless steel.

(Example 157)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void size-gradient conductive member constituted of nickel having numerous voids described in Preparation Example 28 is used instead of the gold-plated foamed stainless steel.

(Example 158)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 159)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 160)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation

Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 161)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of void-containing poly(3,4-ethylenedioxythiophene) described in Preparation Example 6 is used instead of the gold-plated foamed stainless steel.

(Example 162)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void size-gradient conductive member constituted of poly(3,4-ethylenedioxythiophene) having numerous voids described in Preparation Example 29 is used instead of the gold-plated foamed stainless steel.

(Example 163)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void size-gradient conductive member constituted of poly(3,4-ethylenedioxythiophene) having numerous voids described in Preparation Example 29 is used instead of the gold-plated foamed stainless steel.

(Example 164)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void size-gradient conductive member constituted of poly(3,4-

ethylenedioxythiophene) having numerous voids described in Preparation Example 29 is used instead of the gold-plated foamed stainless steel.

(Example 165)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void size-gradient conductive member constituted of poly(3,4-ethylenedioxythiophene) having numerous voids described in Preparation Example 29 is used instead of the gold-plated foamed stainless steel.

(Example 166)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 30 is used instead of the gold-plated foamed stainless steel.

(Example 167)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 30 is used instead of the gold-plated foamed stainless steel.

(Example 168)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive

member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 30 is used instead of the gold-plated foamed stainless steel.

(Example 169)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 30 is used instead of the gold-plated foamed stainless steel.

(Example 170)

An enzyme electrode is prepared in the same manner as in Example 24 except that the conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 30 is used instead of the gold-plated foamed stainless steel.

(Example 171)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void size-gradient conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 31 is used instead of the gold-plated foamed stainless steel.

(Example 172)

An enzyme electrode is prepared in the same

manner as in Example 8 except that the void size-gradient conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 31 is used instead of the gold-plated foamed stainless steel.

(Example 173)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void size-gradient conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 31 is used instead of the gold-plated foamed stainless steel.

(Example 174)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void size-gradient conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 31 is used instead of the gold-plated foamed stainless steel.

(Example 175)

An enzyme electrode is prepared in the same manner as in Example 24 except that the void size-gradient conductive member constituted of gold-plated porous titanium oxide having numerous voids described in Preparation Example 31 is used instead of the gold-plated foamed stainless steel.

(Example 176)

An enzyme electrode is prepared in the same manner as in Example 4 except that the conductive member constituted of nickel alloy having numerous voids described in Preparation Example 32 is used instead of the gold-plated foamed stainless steel. (Example 177)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of nickel alloy having numerous voids described in Preparation Example 32 is used instead of the gold-plated foamed stainless steel. (Example 178)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of nickel alloy having numerous voids described in Preparation Example 32 is used instead of the gold-plated foamed stainless steel. (Example 179)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of nickel alloy having numerous voids described in Preparation Example 32 is used instead of the gold-plated foamed stainless steel. (Example 180)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void size-gradient conductive member constituted of nickel

alloy having numerous voids described in Preparation Example 33 is used instead of the gold-plated foamed stainless steel.

(Example 181)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void size-gradient conductive member constituted of nickel alloy having numerous voids described in Preparation Example 33 is used instead of the gold-plated foamed stainless steel.

(Example 182)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void size-gradient conductive member constituted of nickel alloy having numerous voids described in Preparation Example 33 is used instead of the gold-plated foamed stainless steel.

(Example 183)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void size-gradient conductive member constituted of nickel alloy having numerous voids described in Preparation Example 33 is used instead of the gold-plated foamed stainless steel.

(Example 184)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void size-

gradient conductive member constituted of nickel alloy having numerous voids described in Preparation Example 34 is used instead of the gold-plated foamed stainless steel.

(Example 185)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void size-gradient conductive member constituted of nickel alloy having numerous voids described in Preparation Example 34 is used instead of the gold-plated foamed stainless steel.

(Example 186)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void size-gradient conductive member constituted of nickel alloy having numerous voids described in Preparation Example 34 is used instead of the gold-plated foamed stainless steel.

(Example 187)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void size-gradient conductive member constituted of nickel alloy having numerous voids described in Preparation Example 34 is used instead of the gold-plated foamed stainless steel.

(Example 188)

An enzyme electrode is prepared in the same

manner as in Example 4 except that the conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 35 is used instead of the gold-plated foamed stainless steel.

(Example 189)

An enzyme electrode is prepared in the same manner as in Example 8 except that the conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 35 is used instead of the gold-plated foamed stainless steel.

(Example 190)

An enzyme electrode is prepared in the same manner as in Example 15 except that the conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 35 is used instead of the gold-plated foamed stainless steel.

(Example 191)

An enzyme electrode is prepared in the same manner as in Example 18 except that the conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 35 is used instead of the gold-plated foamed stainless steel.

(Example 192)

An enzyme electrode is prepared in the same manner as in Example 4 except that the void size-gradient conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 36 is used instead of the gold-plated foamed stainless steel.

(Example 193)

An enzyme electrode is prepared in the same manner as in Example 8 except that the void size-gradient conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 36 is used instead of the gold-plated foamed stainless steel.

(Example 194)

An enzyme electrode is prepared in the same manner as in Example 15 except that the void size-gradient conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 36 is used instead of the gold-plated foamed stainless steel.

(Example 195)

An enzyme electrode is prepared in the same manner as in Example 18 except that the void size-gradient conductive member constituted of carbon fiber and having numerous voids described in Preparation Example 36 is used instead of the gold-plated foamed stainless steel.

(Comparative Examples 1 to 26)

Enzyme electrodes are prepared respectively in the same manner as in Examples 1 to 26 except that a gold sheet (1 cm square, 0.3 mm thick, Nilaco) is used as the conductive member instead of the gold-plated foamed stainless steel.

(Example 196)

Sensors are prepared with the enzyme electrodes described in Examples 1 to 195 and Comparative Examples 1 to 26. Fig. 4 shows schematically the three-electrode cell for the measurement. In the cell, the enzyme electrode is employed as the working electrode, an Ag/AgCl electrode is employed as the reference electrode, and a platinum wire is employed as the counter electrode. Into the water-jacketed cell having a cover, air is introduced through a gas tube and a gas inlet. The measurement temperature is kept at 37°C by a constant-temperature water cycling. In the measurement, with the electrodes connected to a potentiostat (Toho Giken K.K., Model 2000), the steady-state current is recorded for the applied potential shown in Table 1. In the electrolytic solution, the electrolyte shown in Table 1 is used corresponding to the substrate for the enzyme of the respective enzyme electrode for the measurement. For measurement with the sensors designated as S12, S13, S21, S25, S67, S68, S104, S105, S137, S138, S157,

S158, S166, and S170 in Table 2, a platinum wire modified by polydimethylsiloxane is used respectively as the counter electrode. For measurement with the sensors designated as S1 to 30, S35 to 38, S118 to 145, and S176 to 195 in Table 2, the enzyme electrodes are prepared as a monolayer electrode as well as a five-layered electrode. All of the sensors employing the enzyme electrode show linear increase of the electric current density with increase of the substrate concentration as exemplified in Figs. 5A, 5B, 6A and 6B, functioning obviously as a sensor. Table 2 shows the electric current densities measured by the sensors.

Table 1

Enzyme	Electrolyte solution	Substrate	Substrate concn mM	Applied voltage V vs Ag/AgCl
Glucose oxidase	1M NaCl 20mM phosphate buffer soln pH 7.2	Glucose	15	0.5
Pyruvate oxidase	15M NaCl 20mM phosphate buffer soln pH 7.4	Oxygen	Saturated	0.2
Laccase	2M citrate buffer soln pH 5.0	Oxygen	Saturated	0.2
Glucose dehydro-genase/ Diaphorase	33mM phosphate buffer soln pH 7.0	Glucose	15	0.5
MP-11	1M phosphate buffer soln pH 7.0	Hydrogen peroxide	1	0
Alcohol dehydrogenase	50mM KCl 50mM Na acetate buffer soln pH 6.0	Ethanol	100	0.5
Cytochrome oxidase	0.1M tris(hydroxymethyl)-aminomethane buffer soln pH 7.0	Oxygen	Saturated	0.2

Table 2

Sym-bol	Enzyme elect-rode	Refe-rence Sen-sor	Current density (monolayer)		Current density (5-layer)	
			Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$	Subst-rate ,Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$
S1	Example 1	S196	18	1000	9	3900
S2	Example 2	S197	8	790	14	3100
S3	Example 3	S198	16	1800	22	6600
S4	Example 4	S199	12	1500	12	5500
S5	Example 5	S200	9	580	11	2200
S6	Example 6	S201	5	470	5	1800
S7	Example 7	S202	42	3600	55	14000
S8	Example 8	S203	13	2800	54	11000
S9	Example 9	S204	4	1500	5	5700
S10	Example 10	S205	2	1100	17	4300
S11	Example 11	S206	18	1100	5	4100
S12	Example 12	S207	14	750	13	2800
S13	Example 13	S208	3	190	1	680
S14	Example 14	S209	9	590	4	2300
S15	Example 15	S210	48	3800	11	14000
S16	Example 16	S211	11	790	15	3000
S17	Example 17	S212	2	370	4	1500
S18	Example 18	S213	13	660	4	2500

Table 2 (Cont'd)

Sym-bol	Enzyme elect-rode	Refe-rence Sen-sor	Current density (monolayer)		Current density (5-layer)	
			Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$	Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$
S19	Example 19	S214	0	310	5	1100
S20	Example 20	S215	5	1200	11	4300
S21	Example 21	S216	1	720	13	2600
S22	Example 22	S217	42	2200	15	8500
S23	Example 23	S218	21	2100	39	7700
S24	Example 24	S219	6	310	4	1200
S25	Example 25	S220	2	190	1	740
S26	Example 26	S221	11	1400	12	5400
S27	Example 27	S199	21	1400	22	5400
S28	Example 28	S203	7	2700	53	10000
S29	Example 29	S210	30	3700	37	14000
S30	Example 30	S213	3	610	4	2400
S31	Example 31	S199	25	1900	-	-
S32	Example 32	S203	47	3500	-	-
S33	Example 33	S210	94	4900	-	-
S34	Example 34	S213	3	840	-	
S35	Example 35	S199	1	390	0.3	1400

Table 2 (Cont'd)

Symbol	Enzyme electrode	Reference Sensor	Current density (monolayer)		Current density (5-layer)	
			Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$	Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$
S36	Example 36	S203	8	680	1	2600
S37	Example 37	S210	9	990	9	3900
S38	Example 38	S213	2	180	2	680
S39	Example 39	S199	10	290	–	–
S40	Example 40	S203	25	540	–	–
S41	Example 41	S210	38	680	–	–
S42	Example 42	S213	8	130	–	–
S43	Example 43	S199	4	380	–	–
S44	Example 44	S203	10	690	–	–
S45	Example 45	S210	12	950	–	–
S46	Example 46	S213	2	180	–	–
S47	Example 47	S219	1	77	–	–
S48	Example 48	S199	8	230	–	–
S49	Example 49	S203	12	420	–	–
S50	Example 50	S210	20	560	–	–
S51	Example 51	S213	2	98	–	–
S52	Example 52	S199	1	370	–	–

Table 2 (Cont'd)

Sym-bol	Enzyme elect-rode	Refe-rence Sen-sor	Current density (monolayer)		Current density (5-layer)	
			Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$	Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$
S53	Example 53	S203	12	720	-	-
S54	Example 54	S210	18	940	-	-
S55	Example 55	S213	0	170	-	-
S56	Example 56	S196	3	580	-	-
S57	Example 57	S197	9	490	-	-
S58	Example 58	S198	1	1000	-	-
S59	Example 59	S199	0	930	-	-
S60	Example 60	S200	2	370	-	-
S61	Example 61	S201	1	280	-	-
S62	Example 62	S202	24	2400	-	-
S63	Example 63	S203	35	1800	-	-
S64	Example 64	S204	4	930	-	-
S65	Example 65	S205	6	680	-	-
S66	Example 66	S206	14	730	-	-
S67	Example 67	S207	0	500	-	-
S68	Example 68	S208	0	110	-	-
S69	Example 69	S209	5	350	-	-

Table 2 (Cont'd)

Symbol	Enzyme electrode	Reference Sensor	Current density (monolayer)		Current density (5-layer)	
			Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$	Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$
S70	Example 70	S210	21	2400	-	-
S71	Example 71	S211	9	480	-	-
S72	Example 72	S212	0	230	-	-
S73	Example 73	S213	8	420	-	-
S74	Example 74	S214	1	190	-	-
S75	Example 75	S215	7	680	-	-
S76	Example 76	S199	2	200	-	-
S77	Example 77	S203	6	390	-	-
S78	Example 78	S210	7	510	-	-
S79	Example 79	S213	2	95	-	-
S80	Example 80	S199	0	260	-	-
S81	Example 81	S203	0	450	-	-
S82	Example 82	S210	8	630	-	-
S83	Example 83	S213	1	120	-	-
S84	Example 84	S199	8	560	-	-
S85	Example 85	S203	19	1100	-	-
S86	Example 86	S210	22	1400	-	-

Table 2 (Cont'd)

Sym-bol	Enzyme elect-rode	Refe-rence Sen-sor	Current density (monolayer)		Current density (5-layer)	
			Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$	Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$
S87	Example 87	S213	3	250	-	-
S88	Example 88	S199	0	280	-	-
S89	Example 89	S203	0	520	-	-
S90	Example 90	S210	11	720	-	-
S91	Example 91	S213	0	130	-	-
S92	Example 92	S219	1	57	-	-
S93	Example 93	S196	4	430	-	-
S94	Example 94	S197	6	320	-	-
S95	Example 95	S198	0	720	-	-
S96	Example 96	S199	10	670	-	-
S97	Example 97	S200	5	260	-	-
S98	Example 98	S201	1	190	-	-
S99	Example 99	S202	11	1700	-	-
S100	Example 100	S203	20	1200	-	-
S101	Example 101	S204	1	640	-	-
S102	Example 102	S205	10	510	-	-
S103	Example 103	S206	10	490	-	-

Table 2 (Cont'd)

Sym-bol	Enzyme elect-rode	Refe-rence Sen-sor	Current density (monolayer)		Current density (5-layer)	
			Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$	Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$
S104	Example 104	S207	4	330	-	-
S105	Example 105	S208	1	79	-	-
S106	Example 106	S209	2	250	-	-
S107	Example 107	S210	11	1600	-	-
S108	Example 108	S211	6	350	-	-
S109	Example 109	S212	1	170	-	-
S110	Example 110	S213	2	310	-	-
S111	Example 111	S214	2	130	-	-
S112	Example 112	S215	1	490	-	-
S113	Example 113	S199	1	330	-	-
S114	Example 114	S203	10	660	-	-
S115	Example 115	S210	5	890	-	-
S116	Example 116	S213	1	160	-	-
S117	Example 117	S219	1	66	-	-
S118	Example 118	S199	3	220	2	880
S119	Example 119	S203	0	440	5	1600
S120	Example 120	S210	1	570	5	2200

Table 2 (Cont'd)

Sym-bol	Enzyme elect-rode	Refe-rence Sen-sor	Current density (monolayer)		Current density (5-layer)	
			Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$	Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$
S121	Example 121	S213	1	110	1	380
S122	Example 122	S199	1	260	2	950
S123	Example 123	S203	4	520	6	2100
S124	Example 124	S210	12	660	4	2500
S125	Example 125	S213	0	120	1	450
S126	Example 126	S196	4	310	4	1200
S127	Example 127	S197	2	240	3	890
S128	Example 128	S198	10	520	9	2000
S129	Example 129	S199	1	460	0.3	1800
S130	Example 130	S200	1	170	2	640
S131	Example 131	S201	2	140	1	510
S132	Example 132	S202	15	1200	16	4500
S133	Example 133	S203	5	870	2	3200
S134	Example 132	S204	2	490	9	1900
S135	Example 135	S205	3	340	3	1300
S136	Example 136	S206	1	370	5	1300
S137	Example 137	S207	3	230	4	820

Table 2 (Cont'd)

Symbol	Enzyme electrode	Reference Sensor	Current density (monolayer)		Current density (5-layer)	
			Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$	Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$
S138	Example 138	S208	0	60	1	240
S139	Example 139	S209	3	180	2	700
S140	Example 140	S210	7	1200	11	4400
S141	Example 141	S211	0	240	5	960
S142	Example 142	S212	0	120	1	440
S143	Example 143	S213	1	210	1	830
S144	Example 144	S214	0	93	1	370
S145	Example 145	S215	4	360	6	1400
S146	Example 146	S199	9	2000	-	-
S147	Example 147	S203	38	4000	-	-
S148	Example 148	S210	88	5300	-	-
S149	Example 149	S213	2	910	-	-
S150	Example 150	S146	14	2300	-	-
S151	Example 151	S147	64	4200	-	-
S152	Example 152	S148	53	5900	-	-
S153	Example 153	S149	5	990	-	-
S154	Example 154	S146	14	2400	-	-

Table 2 (Cont'd)

Symbol	Enzyme electrode	Reference Sensor	Current density (monolayer)		Current density (5-layer)	
			Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$	Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$
S155	Example 155	S147	6	4800	-	-
S156	Example 156	S148	42	6000	-	-
S157	Example 157	S149	12	1200	-	-
S158	Example 158	S199	10	990	-	-
S159	Example 159	S203	11	1800	-	-
S160	Example 160	S210	27	2300	-	-
S161	Example 161	S213	6	420	-	-
S162	Example 162	S158	12	1100	-	-
S163	Example 163	S159	25	2000	-	-
S164	Example 164	S160	44	2800	-	-
S165	Example 165	S161	5	530	-	-
S166	Example 166	S199	2	270	-	-
S167	Example 167	S203	5	530	-	-
S168	Example 168	S210	12	680	-	-
S169	Example 169	S213	2	120	-	-
S170	Example 170	S219	1	57	-	-
S171	Example 171	S166	3	330	-	-

Table 2 (Cont'd)

Symbol	Enzyme electrode	Reference Sensor	Current density (monolayer)		Current density (5-layer)	
			Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$	Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$
S172	Example 172	S167	10	650	-	-
S173	Example 173	S168	16	850	-	-
S174	Example 174	S169	2	150	-	-
S175	Example 175	S170	0	67	-	-
S176	Example 176	S199	32	1700	6	6300
S177	Example 177	S203	5	3100	23	11000
S178	Example 178	S210	13	3900	50	15000
S179	Example 179	S213	3	740	5	2800
S180	Example 180	S176	18	1800	30	7100
S181	Example 181	S177	33	3300	57	13000
S182	Example 182	S178	57	4500	87	17000
S183	Example 183	S179	17	850	10	3400
S184	Example 184	S176	5	2200	33	7900
S185	Example 185	S177	35	4300	36	15000
S186	Example 186	S178	46	5200	46	19000
S187	Example 187	S179	8	1000	3	4100
S188	Example 188	S199	11	1200	22	4600

Table 2 (Cont'd)

Symbol	Enzyme electrode	Reference Sensor	Current density (monolayer)		Current density (5-layer)	
			Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$	Substrate Not added $\mu A/cm^2$	Substrate Added $\mu A/cm^2$
S189	Example 189	S203	12	2400	38	9300
S190	Example 190	S210	54	3000	23	12000
S191	Example 191	S213	9	530	6	2000
S192	Example 192	S188	22	1500	29	5800
S193	Example 193	S189	12	2800	41	11000
S194	Example 194	S190	29	3800	13	15000
S195	Example 195	S191	10	700	5	2800
S196	Comp.Ex. 1		0	120		
S197	Comp.Ex. 2		1	97		
S198	Comp.Ex. 3		1	220		
S199	Comp.Ex. 4		1	180		
S200	Comp.Ex. 5		0	71		
S201	Comp.Ex. 6		1	55		
S202	Comp.Ex. 7		5	480		
S203	Comp.Ex. 8		1	360		
S204	Comp.Ex. 9		0	180		
S205	Comp.Ex. 10		2	150		

Comp.Ex.: Comparative Example

Table 2 (Cont'd)

Sym-bol	Enzyme elect-rode	Refe-rence Sen-sor	Current density (monolayer)		Current density (5-layer)	
			Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$	Subst-rate Not added $\mu A/cm^2$	Subst-rate Added $\mu A/cm^2$
S206	Comp.Ex. 11		1	140		
S207	Comp.Ex. 12		1	91		
S208	Comp.Ex. 13		0	24		
S209	Comp.Ex. 14		1	72		
S210	Comp.Ex. 15		3	490		
S211	Comp.Ex. 16		0	96		
S212	Comp.Ex. 17		0	48		
S213	Comp.Ex. 18		2	90		
S214	Comp.Ex. 19		0	36		
S215	Comp.Ex. 20		1	140		
S216	Comp.Ex. 21		1	85		
S217	Comp.Ex. 22		0	280		
S218	Comp.Ex. 23		2	270		
S219	Comp.Ex. 24		0	39		
S220	Comp.Ex. 25		0	25		
S221	Comp.Ex. 26		1	170		

Comp.Ex.: Comparative Example

Any of the sensors employing the enzyme electrode having a void-containing conductive member in Examples 1 to 149, Examples 158 to 161, Examples 166 to 170, Examples 176 to 179, and Examples 188 to 191 gives a higher current density than that shown by the sensors employing a flat gold electrode, and a corresponding carrier, mediator, enzyme, and substrate. In particular, the sensor having five-layered electrode gives much higher current density, nearly 30-fold at the highest. This shows possibility of increasing the sensitivity of the sensor by use of the void-containing conductive member. Further, the sensors employing the enzyme electrode having a void size-gradient conductive member having numerous voids in Examples 150 to 157, Examples 162 to 165, Examples 171 to 175, Examples 180 to 187, and Examples 192 to 195 give higher current densities than that given by enzyme electrodes of comparative non-void size-gradient conductive members. This shows possibility of further increasing the sensitivity of the sensor by use of a void size-gradient conductive member having numerous voids.

(Example 197)

Fuel cells are produced by use of the enzyme electrodes of Examples with combinations of enzyme electrodes as shown in Table 4, combinations of

electrolytic solutions shown in Table 3, and the kinds and concentrations of the substrates for the enzymes shown in Table 1. Fig. 7 illustrates schematically the two-electrode cell as the measurement reactor. In this reactor, the anode and the cathode with interposition of a porous polypropylene film (20 μm thick) are placed in an electrolytic solution in a water-jacketed capped cell. To the electrolytic solution for the enzyme electrode utilizing oxygen as the substrate, air is fed through a gas tube and a gas inlet. The measurement temperature is kept at 37°C by constant-temperature water cycling. In the measurement, with the electrodes connected to a potentiostat (Toho Giken K.K., Model 2000), the voltage-current characteristics are measured by changing the voltage from -1.2 V to 0.1 V. In the fuel cells employing the enzyme electrode utilizing the enzyme shown in Table 3 as one or both of the electrodes, the electrolytic solution shown in Table 3 is used. In the fuel cells employing none of the enzymes shown in Table 3 for the anode or cathode, the electrolytic solution is a 0.1M NaCl solution in a 20mM phosphate buffer solution saturated with oxygen. For an enzyme electrode containing glucose dehydrogenase/diaphorase or for use of MP-11, an electrochemical measurement is conducted with an electrochemical measurement cell

having a diaphragm (Hokuto Denko K.K.) by separating the anode chamber and the cathode chamber. In the measurements with the fuel cells denoted as FC1-25, FC29-31, FC98-121, and FC145-159, the enzyme electrode is employed as a monolayer as well as a stack of five layers. Table 4 shows the measurement results.

Table 3

Enzyme	Electrolyte solution	Substrate	Substrate concn mM	Applied voltage V vs Ag/AgCl
Laccase	0.2M citrate buffer soln	Oxygen	Saturated	0.2
MP-11	0.1M phosphate buffer soln	Hydrogen peroxide	1	0
Alcohol dehydrogenase	50mM KCl 50mM Na acetate buffer soln	Ethanol	100	0.5
Cytochrome C/ Cytochrome oxidase	0.1M tris (hydroxymethyl)-aminomethane buffer soln	Oxygen	Saturated	0.2

Table 4

Symbol	Anode	Cathode	Reference fuel cell	Short-circuit current density (Monolayer) $\mu A/cm^2$	Maximum power (Monolayer) $\mu W/cm^2$	Short-circuit current density (5-Layer) $\mu A/cm^2$	Maximum power (5-Layer) $\mu W/cm^2$
FC1	Ex 1	Ex 8	FC160	780	70	2700	220
FC2	Ex 2	Ex 8	FC161	590	32	2100	99
FC3	Ex 3	Ex 8	FC162	1300	200	4600	680
FC4	Ex 4	Ex 8	FC163	1300	140	4400	480
FC5	Ex 5	Ex 8	FC164	460	17	1600	53
FC6	Ex 6	Ex 8	FC165	360	6	1200	19
FC7	Ex 9	Ex 8	FC166	1200	130	4300	410
FC8	Ex 10	Ex 8	FC167	960	110	3300	340
FC9	Ex 11	Ex 8	FC168	900	100	3200	340
FC10	Ex 12	Ex 8	FC169	610	190	2100	600
FC11	Ex 13	Ex 8	FC170	150	51	520	160
FC12	Ex 14	Ex 8	FC171	460	0.03	1600	0.08
FC13	Ex 15	Ex 7	FC172	3100	1400	11000	4500
FC14	Ex 15	Ex 20	FC173	3000	660	10000	2100
FC15	Ex 16	Ex 8	FC174	610	18	2100	56
FC16	Ex 17	Ex 8	FC175	320	5	1100	17
FC17	Ex 18	Ex 8	FC176	550	17	1900	54

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC18	Ex 19	Ex 8	FC177	230	4	810	12
FC19	Ex 21	Ex 8	FC178	570	56	2000	170
FC20	Ex 22	Ex 25	FC179	1800	120	6400	370
FC21	Ex 23	Ex 26	FC180	1700	0.09	6000	0.31
FC22	Ex 24	Ex 8	FC181	230	6	810	20
FC23	Ex 27	Ex 28	FC163	1100	130	3800	410
FC24	Ex 29	Ex 28	FC172	2900	870	10000	2800
FC25	Ex 30	Ex 28	FC176	500	14	1800	43
FC26	Ex 31	Ex 32	FC163	1600	170	-	-
FC27	Ex 33	Ex 32	FC172	4000	1200	-	-
FC28	Ex 34	Ex 32	FC176	690	20	-	-
FC29	Ex 35	Ex 36	FC163	290	36	1000	120
FC30	Ex 37	Ex 36	FC172	790	240	2800	750
FC31	Ex 38	Ex 36	FC176	130	4	470	13
FC32	Ex 39	Ex 40	FC163	230	26	-	-
FC33	Ex 41	Ex 40	FC172	570	170	-	-
FC34	Ex 42	Ex 40	FC176	110	3	-	-

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC35	Ex 43	Ex 44	FC163	310	32	-	-
FC36	Ex 45	Ex 44	FC172	770	220	-	-
FC37	Ex 46	Ex 44	FC176	130	3	-	-
FC38	Ex 47	Ex 44	FC181	61	2	-	-
FC39	Ex 48	Ex 47	FC163	180	18	-	-
FC40	Ex 50	Ex 47	FC172	460	130	-	-
FC41	Ex 51	Ex 47	FC176	80	2	-	-
FC42	Ex 52	Ex 53	FC163	300	10	-	-
FC43	Ex 54	Ex 53	FC172	760	65	-	-
FC44	Ex 55	Ex 53	FC176	140	1	-	-
FC45	Ex 56	Ex 63	FC160	490	21	-	-
FC46	Ex 57	Ex 63	FC161	380	11	-	-
FC47	Ex 58	Ex 63	FC162	860	63	-	-
FC48	Ex 59	Ex 63	FC163	790	45	-	-
FC49	Ex 60	Ex 63	FC164	270	6	-	-
FC50	Ex 61	Ex 63	FC165	220	2	-	-
FC51	Ex 64	Ex 63	FC166	740	37	-	-

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) $\mu A/cm^2$	Maxi-mum power (Mono-layer) $\mu W/cm^2$	Short-circuit current density (5-Layer) $\mu A/cm^2$	Maxi-mum power (5-Layer) $\mu W/cm^2$
FC52	Ex 65	Ex 63	FC167	580	37	-	-
FC53	Ex 66	Ex 63	FC168	550	30	-	-
FC54	Ex 67	Ex 63	FC169	360	60	-	-
FC55	Ex 68	Ex 63	FC170	95	15	-	-
FC56	Ex 69	Ex 63	FC171	290	0.009	-	-
FC57	Ex 70	Ex 62	FC172	1800	410	-	-
FC58	Ex 70	Ex 75	FC173	1900	220	-	-
FC59	Ex 71	Ex 63	FC174	390	6	-	-
FC60	Ex 72	Ex 63	FC175	190	2	-	-
FC61	Ex 73	Ex 63	FC176	330	5	-	-
FC62	Ex 74	Ex 63	FC177	140	1	-	-
FC63	Ex 76	Ex 77	FC163	170	4	-	-
FC64	Ex 78	Ex 77	FC172	420	23	-	-
FC65	Ex 79	Ex 77	FC176	76	0	-	-
FC66	Ex 80	Ex 81	FC163	200	3	-	-
FC67	Ex 82	Ex 81	FC172	510	17	-	-

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC68	Ex 83	Ex 81	FC176	88	0.3	-	-
FC69	Ex 84	Ex 85	FC163	440	27	-	-
FC70	Ex 86	Ex 85	FC172	1100	190	-	-
FC71	Ex 87	Ex 85	FC176	210	4	-	-
FC72	Ex 88	Ex 89	FC163	220	10	-	-
FC73	Ex 90	Ex 89	FC172	570	71	-	-
FC74	Ex 91	Ex 89	FC176	110	1	-	-
FC75	Ex 92	Ex 89	FC181	47	1	-	-
FC76	Ex 93	Ex 100	FC160	340	10	-	-
FC77	Ex 94	Ex 100	FC161	270	5	-	-
FC78	Ex 95	Ex 100	FC162	620	29	-	-
FC79	Ex 96	Ex 100	FC163	540	21	-	-
FC80	Ex 97	Ex 100	FC164	190	3	-	-
FC81	Ex 98	Ex 100	FC165	160	1	-	-
FC82	Ex 101	Ex 100	FC166	510	19	-	-
FC83	Ex 102	Ex 100	FC167	400	16	-	-
FC84	Ex 103	Ex 100	FC168	380	14	-	-

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC85	Ex 104	Ex 100	FC169	270	30	-	-
FC86	Ex 105	Ex 100	FC170	66	8	-	-
FC87	Ex 106	Ex 100	FC171	190	0.004	-	-
FC88	Ex 107	Ex 99	FC172	1300	180	-	-
FC89	Ex 107	Ex 112	FC173	1300	100	-	-
FC90	Ex 108	Ex 100	FC174	260	3	-	-
FC91	Ex 109	Ex 100	FC175	130	1	-	-
FC92	Ex 110	Ex 100	FC176	240	2	-	-
FC93	Ex 111	Ex 100	FC177	100	1	-	-
FC94	Ex 113	Ex 114	FC163	260	12	-	-
FC95	Ex 115	Ex 114	FC172	720	88	-	-
FC96	Ex 116	Ex 114	FC176	120	1	-	-
FC97	Ex 117	Ex 114	FC181	53	1	-	-
FC98	Ex 118	Ex 119	FC163	180	4	620	13
FC99	Ex 120	Ex 119	FC172	480	30	1700	92
FC 100	Ex 121	Ex 119	FC176	82	0.5	290	2
FC 101	Ex 122	Ex 123	FC163	210	6	730	18

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC 102	Ex 124	Ex 123	FC172	550	42	1900	140
FC 103	Ex 125	Ex 123	FC176	92	1	320	2
FC 104	Ex 126	Ex 133	FC160	250	6	860	17
FC 105	Ex 127	Ex 133	FC161	180	3	630	8
FC 106	Ex 128	Ex 133	FC162	410	14	1400	40
FC 107	Ex 129	Ex 133	FC163	370	10	1300	34
FC 108	Ex 130	Ex 133	FC164	150	1	510	4
FC 109	Ex 131	Ex 133	FC165	110	1	400	2
FC 110	Ex 134	Ex 133	FC166	380	10	1300	31
FC 111	Ex 135	Ex 133	FC167	280	8	970	26
FC 112	Ex 136	Ex 133	FC 168	300	8	1000	24
FC 113	Ex 137	Ex 133	FC 169	200	16	690	47
FC 114	Ex 138	Ex 133	FC 170	45	4	160	12
FC 115	Ex 139	Ex 133	FC 171	150	0.002	510	0.01
FC 116	Ex 140	Ex 132	FC 172	910	100	3200	330
FC 117	Ex 140	Ex 145	FC 173	970	53	3400	160
FC 118	Ex 141	Ex 133	FC 174	190	1	660	4

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC 119	Ex 142	Ex 133	FC 175	93	0.4	330	1
FC 120	Ex 143	Ex 133	FC 176	180	1	630	4
FC 121	Ex 144	Ex 133	FC 177	79	0.3	280	1
FC 122	Ex 146	Ex 147	FC 163	1700	200	-	-
FC 123	Ex 148	Ex 147	FC 172	4200	1300	-	-
FC 124	Ex 149	Ex 147	FC 176	790	24	-	-
FC 125	Ex 150	Ex 151	FC 163	1900	210	-	-
FC 126	Ex 152	Ex 151	FC 172	4300	1500	-	-
FC 127	Ex 153	Ex 151	FC 176	780	24	-	-
FC 128	Ex 154	Ex 155	FC 163	2000	220	-	-
FC 129	Ex 156	Ex 155	FC 172	4800	1500	-	-
FC 130	Ex 157	Ex 155	FC 176	850	26	-	-
FC 131	Ex 158	Ex 159	FC 163	740	42	-	-
FC 132	Ex 160	Ex 159	FC 172	1900	320	-	-
FC 133	Ex 161	Ex 159	FC 176	330	5	-	-
FC 134	Ex 162	Ex 163	FC 163	930	52	-	-
FC 135	Ex 164	Ex 163	FC 172	2300	370	-	-

Ex: Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC 136	Ex 165	Ex 163	FC 176	400	6	-	-
FC 137	Ex 166	Ex 167	FC 163	220	10	-	-
FC 138	Ex 168	Ex 167	FC 172	590	73	-	-
FC 139	Ex 169	Ex 167	FC 176	100	1	-	-
FC 140	Ex 170	Ex 167	FC 181	44	0.5	-	-
FC 141	Ex 171	Ex 172	FC 163	280	13	-	-
FC 142	Ex 173	Ex 172	FC 172	720	93	-	-
FC 143	Ex 174	Ex 172	FC 176	120	1	-	-
FC 144	Ex 175	Ex 172	FC 181	54	1	-	-
FC 145	Ex 176	Ex 177	FC 163	1200	150	4300	440
FC 146	Ex 178	Ex 177	FC 172	3300	1000	11000	3200
FC 147	Ex 179	Ex 177	FC 176	560	16	2000	53
FC 148	Ex 180	Ex 181	FC 163	1400	170	5000	530
FC 149	Ex 182	Ex 181	FC 172	3700	1200	13000	4100
FC 150	Ex 183	Ex 181	FC 176	660	21	2300	67
FC 151	Ex 184	Ex 185	FC 163	1700	200	5900	620
FC 152	Ex 186	Ex 185	FC 172	4500	1400	16000	4500

Ex: Example

Table 4 (Cont'd)

Symbol	Anode	Cathode	Reference fuel cell	Short-circuit current density (Monolayer) μA/cm2	Maximum power (Monolayer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maximum power (5-Layer) μW/cm2
FC 153	Ex 187	Ex 185	FC 176	780	23	2700	75
FC 154	Ex 188	Ex 189	FC 163	1000	79	3600	250
FC 155	Ex 190	Ex 189	FC 172	2400	530	8400	1700
FC 156	Ex 191	Ex 189	FC 176	450	9	1600	27
FC 157	Ex 192	Ex 193	FC 163	1200	93	4300	290
FC 158	Ex 194	Ex 193	FC 172	3000	610	11000	2000
FC 159	Ex 195	Ex 193	FC 176	530	11	1900	32
FC 160	Comp. Ex 1	Comp. Ex 8		97	10		
FC 161	Comp. Ex 2	Comp. Ex 8		78	5		
FC 162	Comp. Ex 3	Comp. Ex 8		180	28		
FC 163	Comp. Ex 4	Comp. Ex 8		160	21		
FC 164	Comp. Ex 5	Comp. Ex 8		59	3		
FC 165	Comp. Ex 6	Comp. Ex 8		44	1		
FC 166	Comp. Ex 9	Comp. Ex 8		160	18		
FC 167	Comp. Ex 10	Comp. Ex 8		110	14		
FC 168	Comp. Ex 11	Comp. Ex 8		110	13		
FC 169	Comp. Ex 12	Comp. Ex 8		77	28		

Ex: Example Comp.Ex: Comparative Example

Table 4 (Cont'd)

Sym-bol	Ano-de	Ca-th-ode	Re-fer-ence fuel cell	Short-circuit current density (Mono-layer) μA/cm2	Maxi-mum power (Mono-layer) μW/cm2	Short-circuit current density (5-Layer) μA/cm2	Maxi-mum power (5-Layer) μW/cm2
FC 170	Comp. Ex 13	Comp. Ex 8		20	8		
FC 171	Comp. Ex 14	Comp. Ex 8		55	0.003		
FC 172	Comp. Ex 15	Comp. Ex 7		360	180		
FC 173	Comp. Ex 15	Comp. Ex 20		370	93		
FC 174	Comp. Ex 16	Comp. Ex 8		74	2		
FC 175	Comp. Ex 17	Comp. Ex 8		37	1		
FC 176	Comp. Ex 18	Comp. Ex 8		71	2		
FC 177	Comp. Ex 19	Comp. Ex 8		30	1		
FC 178	Comp. Ex 21	Comp. Ex 8		65	7		
FC 179	Comp. Ex 22	Comp. Ex 25		240	20		
FC 180	Comp. Ex 23	Comp. Ex 26		220	0.02		
FC 181	Comp. Ex 24	Comp. Ex 8		29	1		

Comp.Ex: Comparative Example

Any of the fuel cells employing the enzyme electrode having a void-containing conductive member designated in Table 4 as FC1 to 124, FC131 to 133, FC137 to 140, FC145 to 147, and FC154 to 156 gives higher current density than that shown by the fuel

cells employing a flat gold electrode, and a corresponding carrier, mediator, enzyme, and substrate. Most of the fuel cells give a higher maximum power than corresponding fuel cells employing flat gold electrodes. In particular, the sensor having five-layered electrode gives much higher current density, nearly 30-fold at the highest, and the maximum power of nearly 25-fold at the highest. This shows possibility of increasing the output of the fuel cell by use of the void-containing conductive member. Further, the fuel cells employing the enzyme electrode having a void size-gradient conductive member having numerous voids designated as FC125 to 130, FC134 to 136, FC141 to 144, FC148 to 153, and FC157 to 159 give a higher current density and a higher maximum power than that given by enzyme electrodes of comparative non-void size-gradient conductive members. The fuel cells employing the five-layered electrode having a void size-gradient conductive member give a higher current density and a higher maximum power than that given by comparative fuels cells employing non-void size-gradient conductive members. This shows possibility of further increasing the output of the fuel cell by use of a void size-gradient conductive member having numerous voids.

(Example 198)

Flow cell type of fuel cells are constructed with the fuel cells designated as FC1 to 9, FC12 to 18, FC21 to 25, FC29 to 31, FC98 to 112, FC115 to 121, and FC145 to 159 in Table 4. In the flow cells as shown in Fig. 8, five anode-cathode sets are arranged alternately with interposition of porous polypropylene films (thickness: 20 μm, porosity: 80%) in an acrylic resin case. Gold wires of 0.1 mm diameter are connected to the electrodes through the case for electric contact, and fixed to the case with a silicone resin to the case. The measurement is conducted by allowing the electrolytic solution to pass through tubes attached to the acrylic case at a flow rate of 0.25 mL/sec by a precision pump at 37°C. The compositions of the electrolyte solutions are the same as in Example 197. Table 5 shows the measurement results.

Table 5

Symbol	Anode	Cathode	Reference non-flow type fuel cell	Short-circuit current density (Flow type) $\mu A/cm^2$	Maximum power (Flow type) $\mu W/cm^2$
FCF1	Ex 1	Ex 8	FC1	7400	460
FCF2	Ex 2	Ex 8	FC2	6600	270
FCF3	Ex 3	Ex 8	FC3	13000	1300
FCF4	Ex 4	Ex 8	FC4	10000	870
FCF5	Ex 5	Ex 8	FC5	3500	140
FCF6	Ex 6	Ex 8	FC6	2900	38
FCF7	Ex 9	Ex 8	FC7	12000	800
FCF8	Ex 10	Ex 8	FC8	7300	840
FCF9	Ex 11	Ex 8	FC9	8700	740
FCF10	Ex 14	Ex 8	FC12	5300	1200
FCF11	Ex 15	Ex 7	FC13	28000	11000
FCF12	Ex 15	Ex 20	FC14	33000	6600
FCF13	Ex 16	Ex 8	FC15	6100	120
FCF14	Ex 17	Ex 8	FC16	2500	45
FCF15	Ex 18	Ex 8	FC17	4100	140
FCF16	Ex 19	Ex 8	FC18	2300	32
FCF17	Ex 23	Ex 26	FC21	16000	1
FCF18	Ex 24	Ex 8	FC22	2400	45
FCF19	Ex 27	Ex 28	FC23	8600	990
FCF20	Ex 29	Ex 28	FC24	26000	8400
FCF21	Ex 30	Ex 28	FC25	3900	120
FCF22	Ex 35	Ex 36	FC29	2400	260
FCF23	Ex 37	Ex 36	FC30	6700	1900
FCF24	Ex 38	Ex 36	FC31	1000	31
FCF25	Ex 118	Ex 119	FC98	1800	39
FCF26	Ex 120	Ex 119	FC99	4300	210
FCF27	Ex 121	Ex 119	FC100	590	4
FCF28	Ex 122	Ex 123	FC101	2000	57
FCF29	Ex 124	Ex 123	FC102	4100	280
FCF30	Ex 125	Ex 123	FC103	840	5
FCF31	Ex 126	Ex 133	FC104	2300	36
FCF32	Ex 127	Ex 133	FC105	1400	20

Ex: Example

Table 5 (Cont'd)

Symbol	Anode	Cathode	Reference non-flow type fuel cell	Short-circuit current density (Flow type) $\mu A/cm^2$	Maximum power (Flow type) $\mu W/cm^2$
FCF33	Ex 128	Ex 133	FC106	3200	130
FCF34	Ex 129	Ex 133	FC107	3300	110
FCF35	Ex 130	Ex 133	FC108	1200	11
FCF36	Ex 131	Ex 133	FC109	1100	4
FCF37	Ex 134	Ex 133	FC110	3600	83
FCF38	Ex 135	Ex 133	FC111	2100	50
FCF39	Ex 136	Ex 133	FC112	2300	51
FCF40	Ex 139	Ex 133	FC115	1200	0.02
FCF41	Ex 140	Ex 132	FC116	8900	940
FCF42	Ex 140	Ex 145	FC117	7900	520
FCF43	Ex 141	Ex 133	FC118	1700	10
FCF44	Ex 142	Ex 133	FC119	880	3
FCF45	Ex 143	Ex 133	FC120	1500	10
FCF46	Ex 144	Ex 133	FC121	680	3
FCF47	Ex 176	Ex 177	FC145	8900	1100
FCF48	Ex 178	Ex 177	FC146	33000	7400
FCF49	Ex 179	Ex 177	FC147	4100	120
FCF50	Ex 180	Ex 181	FC148	13000	1200
FCF51	Ex 182	Ex 181	FC149	27000	11000
FCF52	Ex 183	Ex 181	FC150	5100	200
FCF53	Ex 184	Ex 185	FC151	17000	1800
FCF54	Ex 186	Ex 185	FC152	46000	13000
FCF55	Ex 187	Ex 185	FC153	6900	210
FCF56	Ex 188	Ex 189	FC154	11000	670
FCF57	Ex 190	Ex 189	FC155	17000	4400
FCF58	Ex 191	Ex 189	FC156	4200	61
FCF59	Ex 192	Ex 193	FC157	12000	710
FCF60	Ex 194	Ex 193	FC158	26000	4600
FCF61	Ex 195	Ex 193	FC159	4700	70

Ex: Example

The flow cell type of fuel cells give higher electric current densities and higher outputs than

that of comparative corresponding non-flow type fuel cells employing the corresponding conductive member, carrier, mediator, enzyme, and substrate shown in Table 4 by a factor of about 2.5. This shows the possibility of increasing the outputs of the fuel cell by constructing the fuel cell in a flow cell type. Among the flow cell type of fuel cells, the void size-gradient fuel cells having numerous voids, FCF50 to 55 and FCF59 to 61, give higher electric current densities and higher outputs than that of comparative corresponding fuel cells having no void-size gradient shown in Table 4. This shows the further possibility of increasing the outputs of the flow type fuel cell by employing the void size-gradient conductive member.

(Example 199)

Electrochemical reactors are constructed with the enzyme electrodes of Examples as shown in Table 6. Three-electrode cells are used in which an enzyme electrode serves as the working electrode, an Ag/AgCl electrode serves as the reference electrode, and a platinum wire serves as the counter electrode as shown in Fig. 4. The electrolytic solution contains 0.1M NaCl, 20mM phosphate buffer, 10mM glucose, and 10mM ethanol. A potential of 0.3 V vs Ag/AgCl is applied for 100 minutes in the water-jacketed cell in a nitrogen atmosphere. The products are

quantitatively determined by high-speed liquid chromatography. In the reactors CR10, CR11, CR18, CR53, CR54, CR83, CR84, CR110, CR111, Cr127, CR128, and CR135 shown in Table 6, the counter electrode is a platinum wire modified by polydimethylsiloxane. Table 6 shows the results.

Table 6

Symbol	Enzyme electrode	Reference reactor	Reaction charge mC	Reaction product	Product quantity μmol
CR1	Ex 1	CR156	5200	Gluconolactone	53
CR2	Ex 2	CR157	4100	Acetaldehyde	41
CR3	Ex 3	CR158	9300	Gluconolactone	89
CR4	Ex 4	CR159	7700	Acetaldehyde	79
CR5	Ex 5	CR160	3300	Gluconolactone	33
CR6	Ex 6	CR161	2500	Acetaldehyde	24
CR7	Ex 9	CR162	8100	Gluconolactone	82
CR8	Ex 10	CR163	5800	Gluconolactone	58
CR9	Ex 11	CR164	6000	Gluconolactone	58
CR10	Ex 12	CR165	4200	Gluconolactone	41
CR11	Ex 13	CR166	990	Gluconolactone	10
CR12	Ex 14	CR167	3100	Gluconolactone	32
CR13	Ex 15	CR168	20000	Gluconolactone	190
CR14	Ex 16	CR169	4500	Gluconolactone	43
CR15	Ex 17	CR170	2000	Gluconolactone	20
CR16	Ex 18	CR171	3700	Gluconolactone	37
CR17	Ex 19	CR172	1600	Gluconolactone	16
CR18	Ex 21	CR173	4100	Gluconolactone	41
CR19	Ex 22	CR174	12000	Gluconolactone	120
CR20	Ex 23	CR175	12000	Gluconolactone	120
CR21	Ex 24	CR176	1700	Gluconolactone	16
CR22	Ex 27	CR159	7500	Acetaldehyde	71
CR23	Ex 29	CR168	20000	Gluconolactone	190
CR24	Ex 30	CR171	3400	Gluconolactone	32
CR25	Ex 31	CR159	9800	Acetaldehyde	95
CR26	Ex 33	CR168	25000	Gluconolactone	240

Ex: Example

Table 6 (Cont'd)

Symbol	Enzyme electrode	Reference reactor	Reaction charge mC	Reaction product	Product quantity μmol
CR27	Ex 34	CR171	4700	Gluconolactone	44
CR28	Ex 35	CR159	2100	Acetaldehyde	20
CR29	Ex 37	CR168	5500	Gluconolactone	57
CR30	Ex 38	CR171	980	Gluconolactone	9
CR31	Ex 39	CR159	1600	Acetaldehyde	16
CR32	Ex 41	CR168	3700	Gluconolactone	37
CR33	Ex 42	CR171	710	Gluconolactone	7
CR34	Ex 43	CR159	2100	Acetaldehyde	21
CR35	Ex 45	CR168	5400	Gluconolactone	52
CR36	Ex 46	CR171	950	Gluconolactone	10
CR37	Ex 47	CR176	410	Gluconolactone	4
CR38	Ex 48	CR159	1300	Acetaldehyde	12
CR39	Ex 50	CR168	3200	Gluconolactone	30
CR40	Ex 51	CR171	550	Gluconolactone	5
CR41	Ex 52	CR159	2000	Acetaldehyde	20
CR42	Ex 54	CR168	5200	Gluconolactone	52
CR43	Ex 55	CR171	870	Gluconolactone	9
CR44	Ex 56	CR156	3000	Gluconolactone	29
CR45	Ex 57	CR157	2500	Acetaldehyde	24
CR46	Ex 58	CR158	5600	Gluconolactone	55
CR47	Ex 59	CR159	5000	Acetaldehdye	49
CR48	Ex 60	CR160	2100	Gluconolactone	21
CR49	Ex 61	CR161	1600	Acetaldehyde	15
CR50	Ex 64	CR162	5100	Gluconolactone	50
CR51	Ex 65	CR163	3800	Gluconolactone	38
CR52	Ex 66	CR164	3900	Gluconolactone	38
CR53	Ex 67	CR165	2800	Gluconolactone	28
CR54	Ex 68	CR166	590	Gluconolactone	6
CR55	Ex 69	CR167	2000	Gluconolactone	19
CR56	Ex 70	CR168	12000	Gluconolactone	120
CR57	Ex 71	CR169	2700	Gluconolactone	26
CR58	Ex 72	CR170	1300	Gluconolactone	12
CR59	Ex 73	CR171	2200	Gluconolactone	22
CR60	Ex 74	CR172	1000	Gluconolactone	10
CR61	Ex 76	CR159	1100	Acetaldehyde	11
CR62	Ex 78	CR168	2900	Gluconolactone	29
CR63	Ex 79	CR171	510	Gluconolactone	5
CR64	Ex 80	CR159	1400	Acetaldehyde	13

Ex: Example

Table 6 (Cont'd)

Symbol	Enzyme electrode	Reference reactor	Reaction charge mC	Reaction product	Product quantity μmol
CR65	Ex 82	CR168	3400	Gluconolactone	35
CR66	Ex 83	CR171	620	Gluconolactone	6
CR67	Ex 84	CR159	3100	Acetaldehyde	31
CR68	Ex 86	CR168	7300	Gluconolactone	70
CR69	Ex 87	CR171	1400	Gluconolactone	13
CR70	Ex 88	CR159	1500	Acetaldehyde	14
CR71	Ex 90	CR168	3800	Gluconolactone	37
CR72	Ex 91	CR171	720	Gluconolactone	7
CR73	Ex 92	CR176	320	Gluconolactone	3
CR74	Ex 93	CR156	2300	Gluconolactone	22
CR75	Ex 94	CR157	1700	Acetaldehyde	17
CR76	Ex 95	CR158	3900	Gluconolactone	38
CR77	Ex 96	CR159	3600	Acetaldehyde	35
CR78	Ex 97	CR160	1400	Gluconolactone	15
CR79	Ex 98	CR161	980	Acetaldehyde	10
CR80	Ex 101	CR162	3400	Gluconolactone	35
CR81	Ex 102	CR163	2600	Gluconolactone	25
CR82	Ex 103	CR164	2700	Gluconolactone	25
CR83	Ex 104	CR165	1700	Gluconolactone	18
CR84	Ex 105	CR166	440	Gluconolactone	4
CR85	Ex 106	CR167	1300	Gluconolactone	12
CR86	Ex 107	CR168	8500	Gluconolactone	88
CR87	Ex 108	CR169	1900	Gluconolactone	18
CR88	Ex 109	CR170	900	Gluconolactone	9
CR89	Ex 110	CR171	1700	Gluconolactone	17
CR90	Ex 111	CR172	690	Gluconolactone	7
CR91	Ex 113	CR159	1800	Acetaldehyde	18
CR92	Ex 115	CR168	4800	Gluconolactone	49
CR93	Ex 116	CR171	830	Gluconolactone	8
CR94	Ex 117	CR176	350	Gluconolactone	3
CR95	Ex 118	CR159	1200	Acetaldehyde	12
CR96	Ex 120	CR168	3100	Gluconolactone	30
CR97	Ex 121	CR171	590	Gluconolactone	6
CR98	Ex 122	CR159	1400	Acetaldehyde	13
CR99	Ex 124	CR168	3400	Gluconolactone	34
CR100	Ex 125	CR171	680	Gluconolactone	7
CR101	Ex 126	CR156	1700	Gluconolactone	16
CR102	Ex 127	CR157	1300	Acetaldehyde	13

Ex: Example

Table 6 (Cont'd)

Symbol	Enzyme electrode	Reference reactor	Reaction charge mC	Reaction product	Product quantity µmol
CR103	Ex 128	CR158	2900	Gluconolactone	29
CR104	Ex 129	CR159	2600	Acetaldehyde	26
CR105	Ex 130	CR160	930	Gluconolactone	9
CR106	Ex 131	CR161	770	Acetaldehyde	7
CR107	Ex 134	CR162	2800	Gluconolactone	27
CR108	Ex 135	CR163	1800	Gluconolactone	18
CR109	Ex 136	CR164	1900	Gluconolactone	20
CR110	Ex 137	CR165	1300	Gluconolactone	13
CR111	Ex 138	CR166	330	Gluconolactone	3
CR112	Ex 139	CR167	960	Gluconolactone	9
CR113	Ex 140	CR168	6700	Gluconolactone	63
CR114	Ex 141	CR169	1200	Gluconolactone	12
CR115	Ex 142	CR170	660	Gluconolactone	7
CR116	Ex 143	CR171	1200	Gluconolactone	11
CR117	Ex 144	CR172	530	Gluconolactone	5
CR118	Ex 146	CR159	11000	Acetaldehyde	100
CR119	Ex 148	CR168	29000	Gluconolactone	280
CR120	Ex 149	CR171	5000	Gluconolactone	47
CR121	Ex 150	CR159	12000	Acetaldehyde	120
CR122	Ex 152	CR168	33000	Gluconolactone	330
CR123	Ex 153	CR171	5600	Gluconolactone	58
CR124	Ex 154	CR159	13000	Acetaldehyde	140
CR125	Ex 156	CR168	31000	Gluconolactone	300
CR126	Ex 157	CR171	6800	Gluconolactone	65
CR127	Ex 158	CR159	5100	Acetaldehyde	51
CR128	Ex 160	CR168	12000	Gluconolactone	110
CR129	Ex 161	CR171	2400	Gluconolactone	24
CR130	Ex 162	CR159	6200	Acetaldehyde	62
CR131	Ex 164	CR168	15000	Gluconolactone	150
CR132	Ex 165	CR171	2700	Gluconolactone	26
CR133	Ex 166	CR159	1400	Gluconolactone	14
CR134	Ex 168	CR168	3700	Acetaldehyde	38
CR135	Ex 169	CR171	650	Gluconolactone	7
CR136	Ex 170	CR176	300	Gluconolactone	3
CR137	Ex 171	CR159	1800	Acetaldehyde	17
CR138	Ex 173	CR168	4700	Gluconolactone	46
CR139	Ex 174	CR171	820	Gluconolactone	9
CR140	Ex 175	CR176	370	Gluconolactone	4

Ex: Example

Table 6 (Cont'd)

Symbol	Enzyme electrode	Reference reactor	Reaction charge mC	Reaction product	Product quantity μmol
CR141	Ex 176	CR159	9200	Acetaldehyde	87
CR142	Ex 178	CR168	20000	Gluconolactone	190
CR143	Ex 179	CR171	4000	Gluconolactone	40
CR144	Ex 180	CR159	9800	Acetaldehyde	94
CR145	Ex 182	CR168	24000	Gluconolactone	240
CR146	Ex 183	CR171	4400	Gluconolactone	43
CR147	Ex 184	CR159	12000	Acetaldehyde	110
CR148	Ex 186	CR168	27000	Gluconolactone	270
CR149	Ex 187	CR171	5500	Gluconolactone	56
CR150	Ex 188	CR159	6500	Acetaldehyde	64
CR151	Ex 190	CR168	16000	Gluconolactone	150
CR152	Ex 191	CR171	2900	Gluconolactone	29
CR153	Ex 192	CR159	7900	Acetalsehdye	75
CR154	Ex 194	CR168	20000	Gluconolactone	190
CR155	Ex 195	CR171	3600	Gluconolactone	37
CR156	Comp.Ex 1		620	Gluconolactone	6
CR157	Comp.Ex 2		520	Acetaldehyde	5
CR158	Comp.Ex 3		1200	Gluconolactone	12
CR159	Comp.Ex 4		970	Acetaldehyde	10
CR160	Comp.Ex 5		390	Gluconolactone	4
CR161	Comp.Ex 6		300	Acetaldehyde	3
CR162	Comp.Ex 9		980	Gluconolactone	9
CR163	Comp.Ex 10		840	Gluconolactone	8
CR164	Comp.Ex 11		720	Gluconolactone	7
CR165	Comp.Ex 12		480	Gluconolactone	5
CR166	Comp.Ex 13		120	Gluconolactone	1

Ex: Example Comp.Ex: Comparative Example

Table 6 (Cont'd)

Symbol	Enzyme electrode	Reference reactor	Reaction charge mC	Reaction product	Product quantity μmol
CR167	Comp.Ex 14		380	Gluconolactone	4
CR168	Comp.Ex 15		2600	Gluconolactone	27
CR169	Comp.Ex 16		520	Gluconolactone	5
CR170	Comp.Ex 17		270	Gluconolactone	3
CR171	Comp.Ex 18		500	Gluconolactone	5
CR172	Comp.Ex 19		200	Gluconolactone	2
CR173	Comp.Ex 21		470	Gluconolactone	5
CR174	Comp.Ex 22		1500	Gluconolactone	14
CR175	Comp.Ex 23		1400	Gluconolactone	14
CR176	Comp.Ex 24		210	Gluconolactone	2

Comp.Ex: Comparative Example

From the reaction solution of the reactor employing an enzyme electrode having an enzyme utilizing glucose as the substrate (glucose oxidase, and glucose dehydrogenase), gluconolactone is detected without detection of acetaldehyde. From the reaction solution of the reactor employing an enzyme electrode having an enzyme utilizing an alcohol as the substrate (alcohol dehydrogenase), acetaldehyde is detected without detection of gluconolactone. Thus in any of the reactor employing the enzyme

electrode, the reaction proceeds selectively with the substrate. Further, in any of the reactor, the reaction charge quantity and the formed substance are in high correlation, showing the quantitativeness of the reaction. With the reactors CR1 to 120, CR127 to 129, CR133 to 136, CR141 to 143, and CR150 to 152 in Table 6 employing the enzyme electrode with the void-containing conductive member give larger reaction charge quantity than the comparative reactors employing a flat gold electrode with the corresponding carrier, mediator, enzyme, and substrate. This shows possibility of shortening of the reaction time by use of the void-containing conductive member. Further, the chemical reactors employing the enzyme electrode having a void size-gradient conductive member having numerous voids denoted in Table 6 as CR121 to 126, CR130 to 132, CR137 to 140, CR144 to 149, and CR153 to 155 give a larger reaction charge quantity and a larger product quantity than the comparative apparatuses employing a conductive member having no void-size gradient. This shows the possibility of further shortening of the reaction time by use of the void size-gradient conductive member.

(Example 200)

Flow cell type reactors are constructed with the electrochemical reactors designated as CR1 to 9,

CR12 to 17, CR19 to 24, CR28 to 30, CR95 to 109, CR112 to 117, and CR141 to 155 in the above Table. In the flow cell, an enzyme electrode is employed as the working electrode, a platinum net (Nilaco, 150 mesh) is employed as the counter electrode. As shown in Fig. 8, five sets of a working electrodes and a counter electrode are arranged alternately with interposition of porous polypropylene films (thickness: 20 μm, porosity: 80%) in an acrylic case. Gold wires of 0.1 mm diameter are connected to the electrodes through the case for electric contact, and fixed to the case with a silicone resin to the case. The measurement is conducted by allowing the electrolytic solution to circulate through tubes attached to holes of the acrylic case at a flow rate of 0.5 mL/sec by a precision pump at 37°C. The electrolytic solution contains 0.1M NaCl, 20mM phosphate buffer, 10mM glucose, and 10mM ethanol. In a nitrogen atmosphere, a voltage of 1.5 V is applied for 100 minutes. The products are quantitatively determined by high-speed liquid chromatography. Table 7 shows the results.

Table 7

Symbol	Enzyme electrode	Reference non-flow type reactor	Reaction charge Mc	Reaction product	Product quantity μmol
CRF1	Ex 1	CR1	53000	Gluconolactone	540
CRF2	Ex 2	CR2	53000	Acetaldehyde	500
CRF3	Ex 3	CR3	130000	Gluconolactone	1300
CRF4	Ex 4	CR4	100000	Acetaldehyde	990
CRF5	Ex 5	CR5	42000	Gluconolactone	410
CRF6	Ex 6	CR6	29000	Acetaldehyde	270
CRF7	Ex 9	CR7	88000	Gluconolactone	910
CRF8	Ex 10	CR8	78000	Gluconolactone	800
CRF9	Ex 11	CR9	72000	Gluconolactone	680
CRF10	Ex 14	CR12	49000	Gluconolactone	490
CRF11	Ex 15	CR13	12000	Gluconolactone	110
CRF12	Ex 16	CR14	34000	Gluconolactone	340
CRF13	Ex 17	CR15	230000	Gluconolactone	2300
CRF14	Ex 18	CR16	48000	Gluconolactone	460
CRF15	Ex 19	CR17	21000	Gluconolactone	200
CRF16	Ex 22	CR19	39000	Gluconolactone	370
CRF17	Ex 23	CR20	19000	Gluconolactone	180
CRF18	Ex 24	CR21	56000	Gluconolactone	530
CRF19	Ex 27	CR22	140000	Acetaldehyde	1300
CRF20	Ex 29	CR23	150000	Gluconolactone	1500
CRF21	Ex 30	CR24	19000	Gluconolactone	190
CRF22	Ex 35	CR28	88000	Acetaldehyde	830
CRF23	Ex 37	CR29	270000	Gluconolactone	2800
CRF24	Ex 38	CR30	36000	Gluconolactone	340
CRF25	Ex 118	CR95	130000	Acetaldehyde	1200
CRF26	Ex 120	CR96	350000	Gluconolactone	3500
CRF27	Ex 121	CR97	52000	Gluconolactone	520
CRF28	Ex 122	CR98	24000	Acetaldehyde	240
CRF29	Ex 124	CR99	61000	Gluconolactone	600

Ex: Example

Table 7 (Cont'd)

Symbol	Enzyme electrode	Reference non-flow type reactor	Reaction charge Mc	Reaction product	Product quantity μmol
CRF30	Ex 125	CR100	12000	Gluconolactone	120
CRF31	Ex 126	CR101	17000	Gluconolactone	170
CRF32	Ex 127	CR102	42000	Acetaldehyde	420
CRF33	Ex 128	CR103	9300	Gluconolactone	95
CRF34	Ex 129	CR104	29000	Acetaldehyde	290
CRF35	Ex 130	CR105	61000	Gluconolactone	620
CRF36	Ex 131	CR106	11000	Acetaldehyde	110
CRF37	Ex 134	CR107	4200	Gluconolactone	40
CRF38	Ex 135	CR108	14000	Gluconolactone	140
CRF39	Ex 136	CR109	44000	Gluconolactone	450
CRF40	Ex 139	CR112	6100	Gluconolactone	58
CRF41	Ex 140	CR113	25000	Gluconolactone	240
CRF42	Ex 141	CR114	69000	Gluconolactone	700
CRF43	Ex 142	CR115	9400	Gluconolactone	96
CRF44	Ex 143	CR116	41000	Gluconolactone	400
CRF45	Ex 144	CR117	31000	Gluconolactone	310
CRF46	Ex 176	CR141	110000	Acetaldehyde	1000

Ex: Example

Table 7 (Cont'd)

Symbol	Enzyme electrode	Reference non-flow type reactor	Reaction charge Mc	Reaction product	Product quantity μmol
CRF47	Ex 178	CR142	240000	Gluconolactone	2400
CRF48	Ex 179	CR143	47000	Gluconolactone	470
CRF49	Ex 180	CR144	99000	Acetaldehyde	990
CRF50	Ex 182	CR145	250000	Gluconolactone	2400
CRF51	Ex 183	CR146	53000	Gluconolactone	530
CRF52	Ex 184	CR147	140000	Acetaldehyde	1400
CRF53	Ex 186	CR148	290000	Gluconolactone	2900
CRF54	Ex 187	CR149	58000	Gluconolactone	590
CRF55	Ex 188	CR150	87000	Acetaldehyde	830
CRF56	Ex 190	CR151	190000	Gluconolactone	2000
CRF57	Ex 191	CR152	30000	Gluconolactone	290
CRF58	Ex 192	CR153	85000	Acetaldehyde	810
CRF59	Ex 194	CR154	210000	Gluconolactone	2100
CRF60	Ex 195	CR155	45000	Gluconolactone	440

Ex: Example

The flow cell type electrochemical reactor gives a larger reaction charge quantity and a larger reaction product quantity than the comparative

corresponding ones shown in Table 6 employing a corresponding conductive member, carrier, mediator, enzyme, and substrate by a factor of nearly 3. This shows the possibility of shortening of the reaction time by the flow cell structure. Further, among the chemical reactors of the flow cell structure, the chemical reactors employing a void size-gradient conductive member having numerous voids designated as CRF49 to 54 and CRF58 to 60 give a larger reaction charge quantity and a larger product quantity than the comparative corresponding fuel cells having no void-size gradient. This shows possibility of still further shortening the reaction time by use of the void size-gradient conductive member with the flow cell type of the chemical reactor.

This application claims priority from Japanese Patent Application Nos. 2004-216287 filed July 23, 2004 and 2005-023520 filed January 31, 2005, which are hereby incorporated by reference herein.

CLAIMS

1. An enzyme electrode having a conductive member and an enzyme, wherein the conductive member has a porous structure, and the enzyme is immobilized through a carrier in pores constituting the porous structure.

2. The enzyme electrode according to claim 1, wherein the size of the pores on the surface side of porous structure of the conductive member is larger than the size of the pores in the interior of the conductive member.

3. The enzyme electrode according to claim 1, wherein the enzyme electrode contains a mediator for promoting transfer of electrons between the enzyme and the conductive member.

4. The enzyme electrode according to claim 1 or 2, wherein the conductive member comprises at least one of materials selected from metals, conductive polymers, metal oxides, and carbonaceous materials.

5. The enzyme electrode according to claim 1, wherein the enzyme is a redox enzyme.

6. The enzyme electrode according to claim 1, wherein the conductive member has at least two working faces opposing each other, and a liquid is permeable through the numerous voids between the two faces.

7. An enzyme electrode device, comprising the enzyme electrode set forth in Claim 6, and wiring connected to the conductive member of the enzyme electrode.

8. The enzyme electrode device according to claim 7, wherein plural enzyme electrodes are laminated with the working faces thereof opposed.

9. A sensor, employing the enzyme electrode device set forth in claim 7 or 8 as a detector for detecting a substance.

10. A fuel cell having an anode and a cathode, and a region for retaining an electrolytic solution between the anode and cathode, wherein at least one of the anode and the cathode is the enzyme electrode device set forth in claim 7 or 8.

11. An electrochemical reactor having a reaction region, and an electrode for causing an electrochemical reaction of a source material introduced to the reaction region, wherein the electrode is the enzyme electrode device set forth in claim 7 or 8.

12. A process for producing an enzyme electrode, comprising steps of:

providing a conductive member having numerous voids communicating with each other and communicating with the outside, and a carrier for immobilizing an enzyme for transfer of electrons to or from the conductive

member; and

immobilizing the enzyme in the voids with immobilization of the carrier in the voids.

13. A fuel cell, wherein an anode and a cathode have a porous structure, and at least one of the anode and the cathode is an enzyme electrode having an enzyme in pores constituting the porous structure.

14. The fuel cell according to claim 13, wherein the size of the pores on the surface side of the enzyme structure is larger than the size of the pores in the interior of the enzyme electrode.

FIG. 1

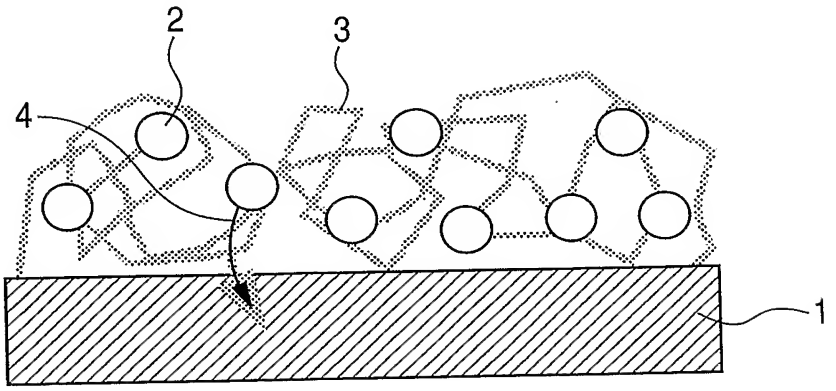


FIG. 2

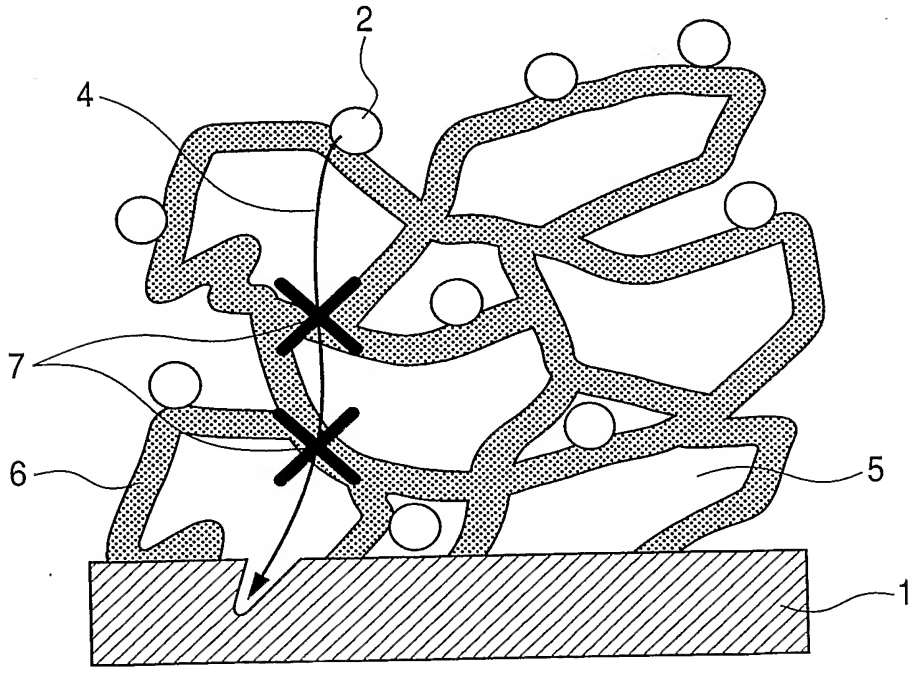


FIG. 3

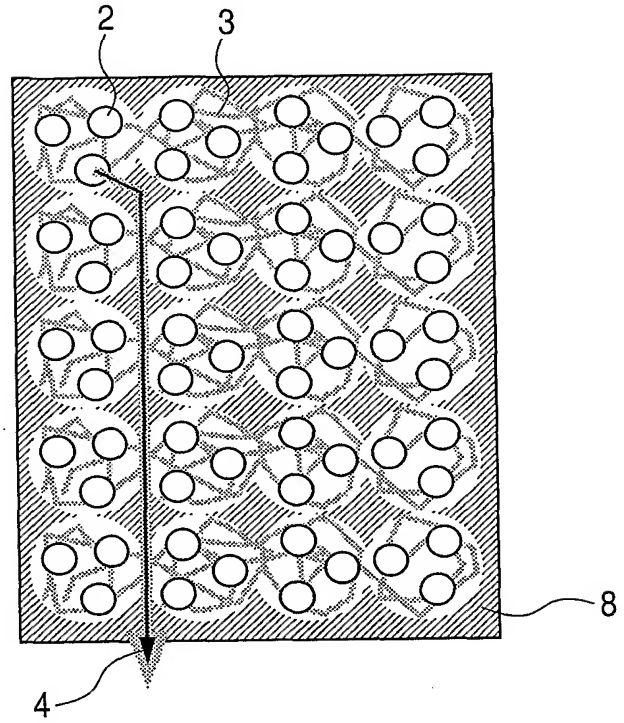


FIG. 4

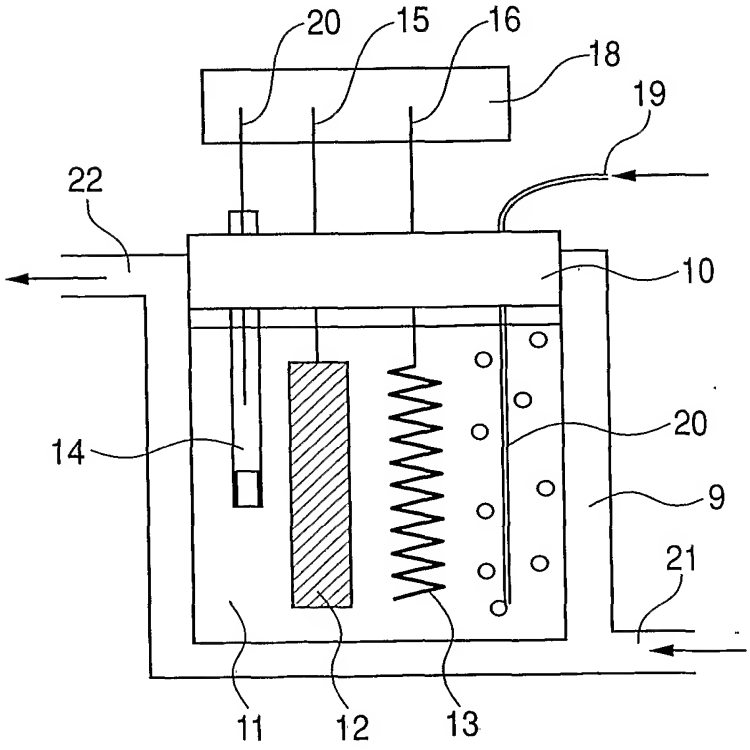


FIG. 5A

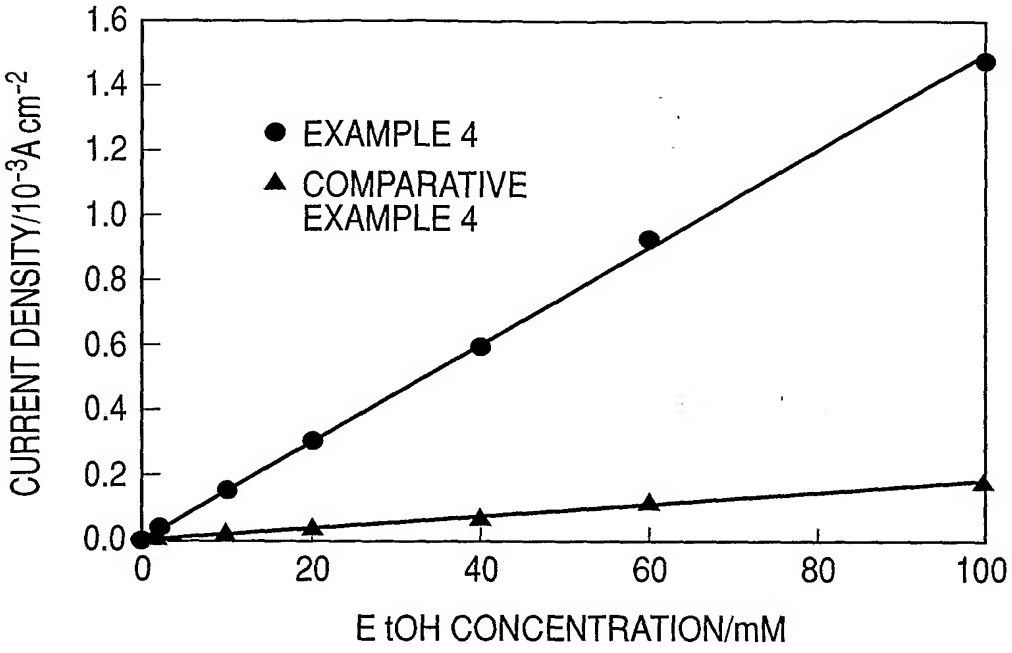

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CURRENT DENSITY/10-3A cm-2
EXAMPLE 4
COMPARATIVE EXAMPLE 4
0
20
40
60
80
100
E tOH CONCENTRATION/mM

FIG. 5B

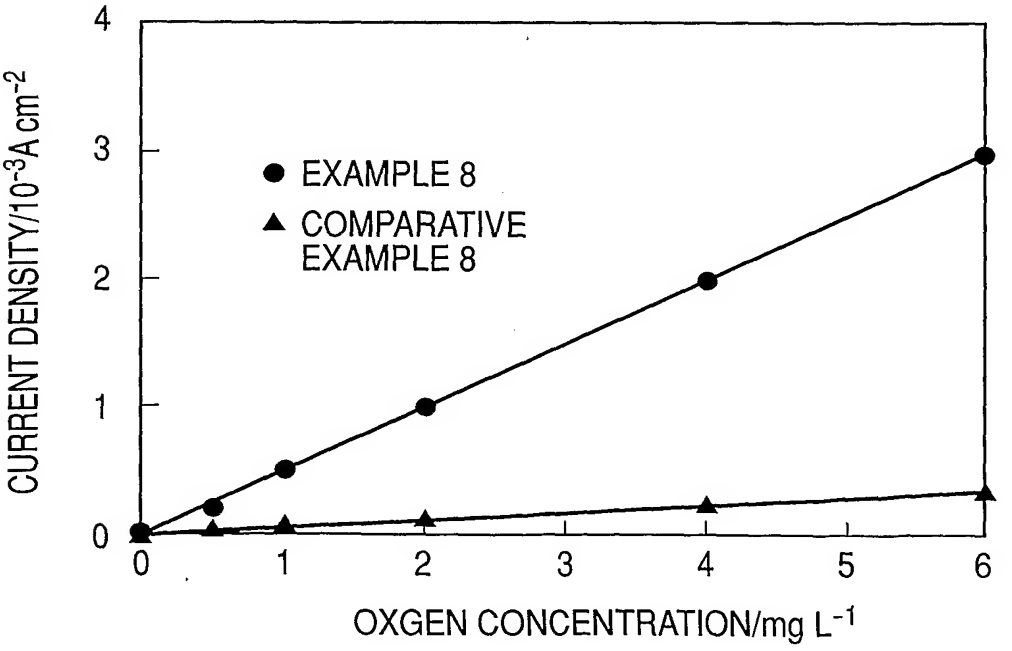

4
3
2
1
0
CURRENT DENSITY/10-3A cm-2
EXAMPLE 8
COMPARATIVE EXAMPLE 8
0
1
2
3
4
5
6
OXGEN CONCENTRATION/mg L-1

FIG. 6A

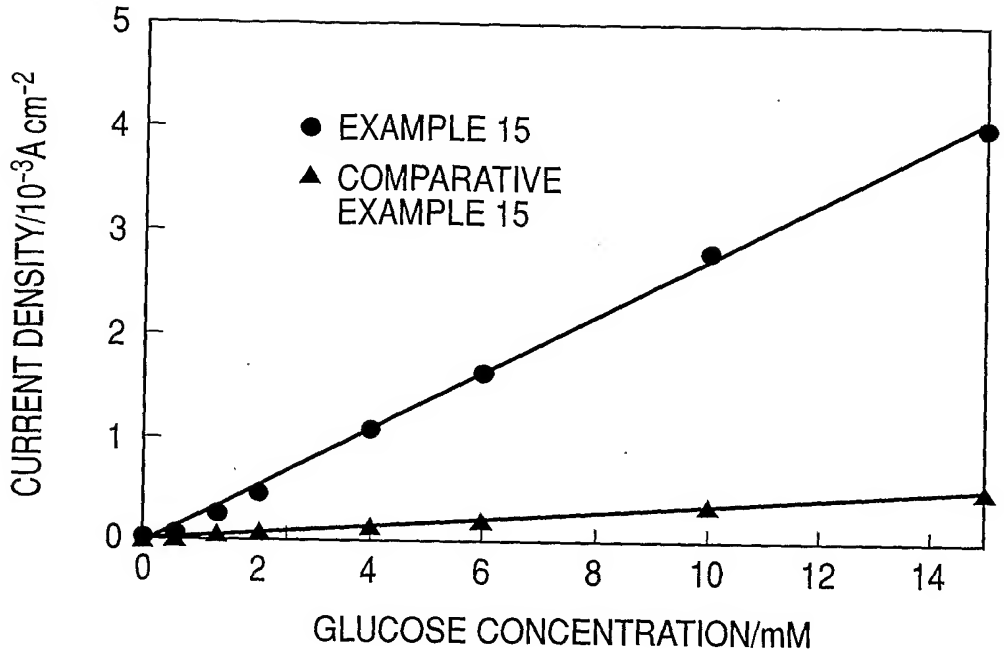

CURRENT DENSITY/10^{-3}A cm^{-2}
5
4
3
2
1
0
EXAMPLE 15
COMPARATIVE EXAMPLE 15
0
2
4
6
8
10
12
14
GLUCOSE CONCENTRATION/mM

FIG. 6B

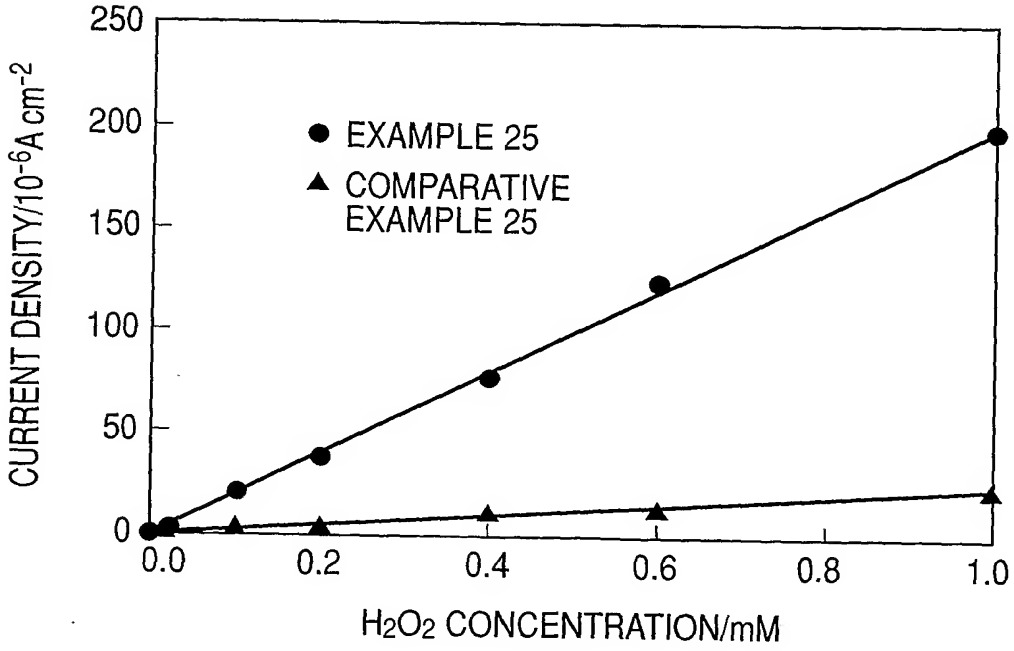

CURRENT DENSITY/10^{-6}A cm^{-2}
250
200
150
100
50
0
EXAMPLE 25
COMPARATIVE EXAMPLE 25
0.0
0.2
0.4
0.6
0.8
1.0
H_2O_2 CONCENTRATION/mM

FIG. 7

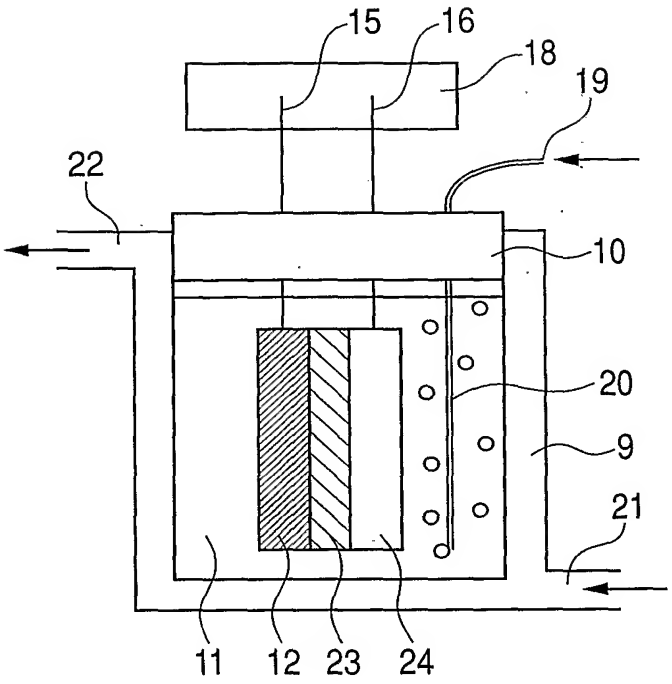


FIG. 8

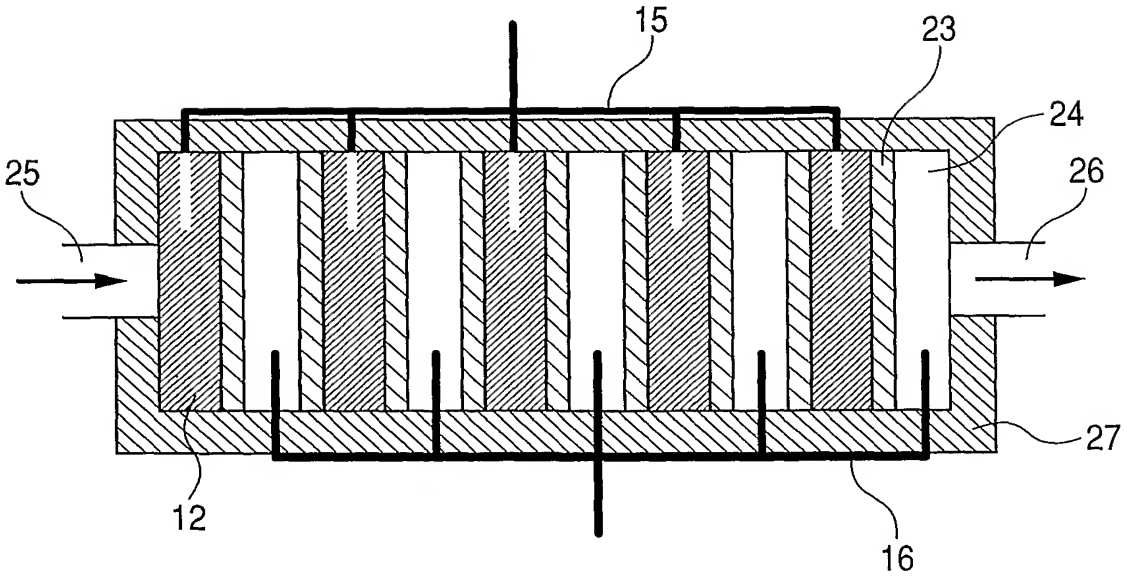


FIG. 9A

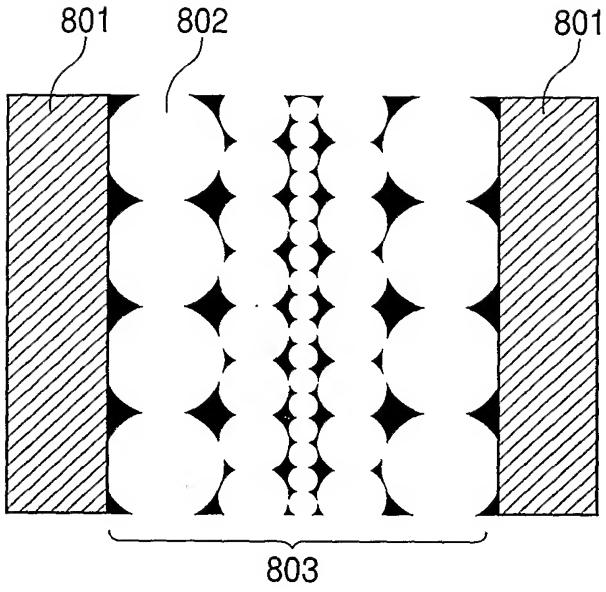


FIG. 9B

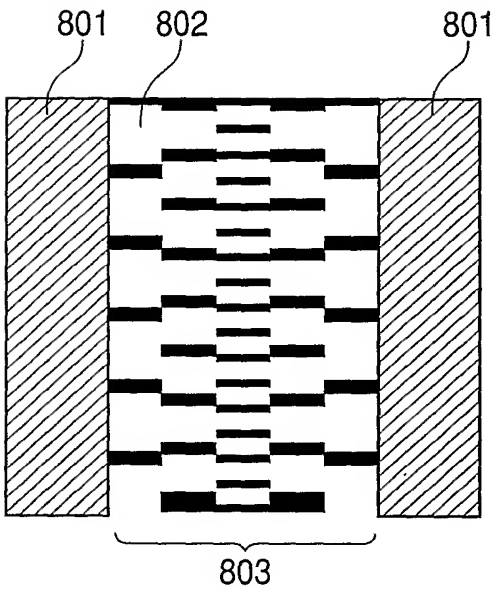


FIG. 9C

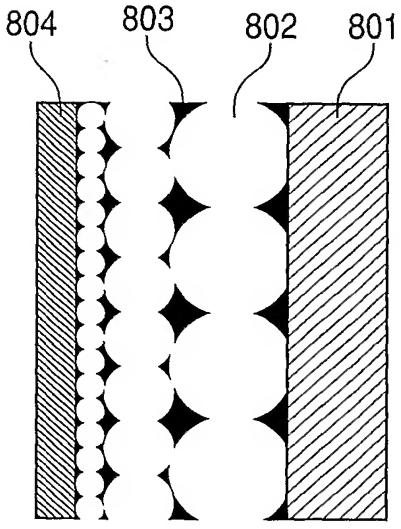
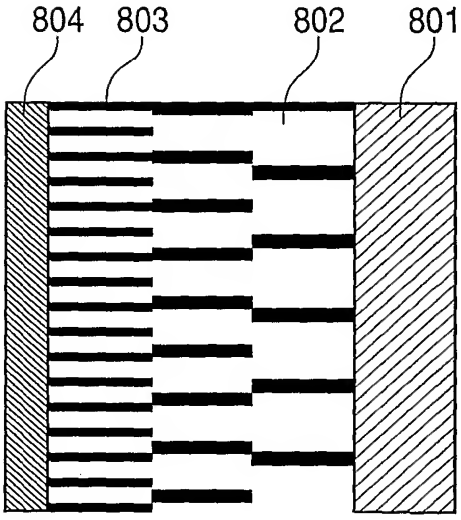


FIG. 9D



INTERNATIONAL SEARCH REPORT

Interi ıal Application No
PCT/JP2005/013896

A. CLASSIFICATION OF SUBJECT MATTER
G01N27/327 C12Q1/00 C12N11/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Z. ZHANG ET AL: "Electrochemical fabrication of amperometric glucose enzyme electrode by immobilizing glucose oxidase in electropolymerized poly (3,3'-diaminobenzidine) film on palladinized glassy carbon electrode." ANALYST, vol. 121, July 1996 (1996-07), pages 971-976, XP008055777 page 972	1,3-5,12
X	US 5 269 903 A (IKARIYAMA ET AL) 14 December 1993 (1993-12-14)	1,4,5,12
A	column 5, lines 31-39 - column 6, lines 3-9,47-57	6-12

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
18 November 2005	07/12/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreno, C

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/JP2005/013896

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/101741 A1 (MINTEER SHELLEY D ET AL) 27 May 2004 (2004-05-27) page 3, column 1, paragraph 37 - column 2, paragraph 39	13
A	US 4 970 145 A (BENNETTO ET AL) 13 November 1990 (1990-11-13) cited in the application abstract	1,12
A	US 5 283 186 A (CUNNINGHAM ET AL) 1 February 1994 (1994-02-01) abstract	1

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Information on patent family members

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